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The Role of the Interleukin-6 Pathway in Asthma

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ABSTRACT

Asthma is a chronic airway disease with high prevalence worldwide. Symptoms – including wheeze, cough, shortness of breath and chest tightness – can be life-threatening and long-lasting. Yet, asthma remains without cure, and the need for improved treatments is clear. In a genome-wide association study (GWAS) performed in 2011,[1] a variant (rs4129267) in the interleukin-6 (IL-6) receptor gene (*IL6R*) was reported to associate with asthma risk. That study showed that asthmatics are more likely to carry the rs4129267:T allele that correlates with increased serum levels of the soluble IL-6 receptor (sIL-6R), which in turn appears to extend the inflammatory effects of IL-6 through a pathway called trans-signalling. Subsequently, a variant in high linkage disequilibrium with rs4129267:T was shown to associate with poor lung function and severe asthma. Together with functional studies that implicated the IL-6 pathway in asthma, these findings suggested that anti-IL-6R therapy – specifically inhibition of IL-6 trans-signalling – might represent a new treatment option for asthma.

The goal of this thesis was to study that hypothesis and to understand the significance of the IL-6 pathway in asthma. Specifically, four main questions were addressed:

1. What characterises asthma patients likely to benefit from anti-IL-6R therapy?
2. Is IL-6 trans-signalling more likely to be promoted by some allergen types?
3. Are there other genes that associate with IL-6 signalling that are also risk factors for asthma?
4. Can asthma symptoms be attenuated by a drug that blocks IL-6 signalling?

To address the first question (Chapter 2), sputum IL-6 and sIL-6R levels were tested for association with relevant clinical information available for 33 asthmatics. Sputum levels of IL-6 and sIL-6R were largely independent of levels measured in matched serum samples. On the other hand, sputum levels of IL-6 and sIL-6R associated with the total number of immune cells measured in the same samples. High sputum levels of IL-6 and sIL-6R – which promotes IL-6 trans-signalling – were more likely to be observed in patients with neutrophilic and mixed granulocytic sputum inflammatory subtypes.

To assess whether some allergen types were more likely to trigger airway inflammation that is associated with IL-6 trans-signalling (Chapter 3), the frequency of sputum subtypes observed after allergen challenge was studied in 129 asthma patients exposed to different allergen types. Overall, baseline sputum inflammatory subtypes shifted towards eosinophilic and mixed granulocytic

inflammation after allergen inhalation. However, no difference was observed in the frequency of sputum inflammatory subtypes between the different allergen types used in the allergen challenge.

In Chapter 4, data from gene expression studies and asthma GWAS were analysed to study gene networks associated with IL-6 signalling in asthma. Specifically, genes co-expressed with *IL6R* were screened for nearby variants that associated both with the expression of that gene and asthma risk. Five such genes were identified, including one which had not previously been implicated in disease pathophysiology: *STOML2*. This gene plays a key role in mitochondrial function and T-cell activation. Follow-up analyses (Chapter 5) of the *STOML2* risk variant showed that it has a stronger effect in early-onset asthma, while it also associates with the risk of hay fever.

Finally, two approaches were used to determine if tocilizumab – a drug that blocks IL-6 classic and trans-signalling and is approved to treat rheumatoid arthritis – represents a promising new treatment for asthma.

First, data from a proof-of-concept clinical trial were analysed to assess the effect of a single dose of tocilizumab on allergen-induced asthma exacerbations (Chapter 6). Eleven participants with mild asthma completed the trial. Lung function, airway hyperresponsiveness, pathology results and inflammatory cells and mediators were compared between the tocilizumab and placebo groups. Overall, the drug did not inhibit allergen-induced airway responses. One participant with mixed-granulocytic sputum, rs4129267:TT genotype and treated with tocilizumab did not develop a late asthmatic response, but this should not be over-interpreted. Second, using data from the Australian Pharmaceutical Benefits Scheme (Chapter 7), individuals who were treated regularly with tocilizumab and were also likely to suffer from asthma were identified (N = 88). Then, in these individuals and a group of matched controls (N = 440), the number of asthma-related prescriptions was compared one year before against one year after the onset of tocilizumab treatment. Results showed no significant differences in the number and frequency of inhaled corticosteroids, suggesting that regular tocilizumab is unlikely to have an effect in asthma symptoms.

In summary, (1) IL-6 trans-signalling was associated with mixed-granulocytic sputum; (2) different allergens were unlikely to influence sputum inflammatory subtype; (3) *STOML2* was identified as an *IL6R*-associated gene with suggestive association with early-onset allergic disease; and (4) tocilizumab treatment did not reduce allergen-induced bronchoconstriction, nor the frequency of asthma-related prescriptions.

DECLARATION BY AUTHOR

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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PUBLICATIONS DURING CANDIDATURE

1. Ullah MA, **Revez JA**, Loh Z, Simpson J, Zhang V, Bain L, Varelias A, Rose-John S, Blumenthal A, Smyth MJ, Hill GR, Sukkar MB, Ferreira MA, Phipps S: Allergen-induced IL-6 trans-signaling activates gammadelta T cells to promote type 2 and type 17 airway inflammation. J Allergy Clin Immunol 2015;136:1065-1073.

2. **Revez JA**, Matheson MC, Hui J, Baltic S, Australian Asthma Genetics Consortium c, James A, Upham JW, Dharmage S, Thompson PJ, Martin NG, Hopper JL, Ferreira MA: Identification of STOML2 as a putative novel asthma risk gene associated with IL6R. Allergy 2016;71:1020-1030.

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Chapter 6 includes results from a clinical trial to which the following contributions were made:

- Porf. Gail M Gauvreau: led the clinical trial site at McMaster University, being responsible for ethical and regulatory approval, supervision of participant recruitment and clinical testing. She also developed and provided the clinical protocol used in the trial and trained the Brisbane team.
- Prof. Paul O'Byrne: Developed the clinical protocol used in the trial.
- Mr. Rick Watson: recruited and clinically tested all the participants at McMaster University.
- Ms. Lisa Bain: Responsible for ethics and regulatory approval of the trial protocol in Brisbane, sourcing all the material and equipment for the trial, recruiting participants, clinical testing,

assisting clinical nurses at both Brisbane sites, measuring inflammatory mediators in biological samples.

- Ms. Michelle Towers: clinical nurse at the Princess Alexandra Hospital (Brisbane) who was responsible for all clinical procedures involving drug administration.
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- Dr. Manuel Ferreira: led the clinical trial site at Q-Pharm and Princess Alexandra Hospital, being responsible for ethical and regulatory approval, supervision of participant recruitment and clinical testing.
- Prof. John Upham: clinical leader of the tocilizumab trial, responsible for all clinical decisions at the Brisbane sites and direct supervision of clinical trial staff at the Princess Alexandra Hospital

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STATEMENT OF PARTS OF THE THESIS SUBMITTED TO QUALIFY FOR THE AWARD OF ANOTHER DEGREE

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RESEARCH INVOLVING HUMAN OR ANIMAL SUBJECTS

Research projects included in this thesis that involved human subjects were performed under the following ethics approvals:

- Chapter 2. Clinical features of patients with a microenvironment in the airways that promotes IL-6 trans-signalling – **Project P710**
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- Chapter 6. Prevention of allergen-induced asthma exacerbations with tocilizumab – **Projects P2025, P2103 and 14-790**
- Chapter 7. Frequency of asthma prescriptions in patients on regular tocilizumab treatment – **Project P2242**

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LIST OF ABBREVIATIONS IN THE THESIS

ACQ	Asthma Control Questionnaire
ADA	Adalimumab
AE	Adverse event
AIHW	Australian Institute of Health and Welfare
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
CNTF	Ciliary Neurotrophic Factor
COPD	Chronic obstructive pulmonary disease
DMAP	Differentiation Map Portal
DTT	Dithiothreitol

EAR	Early asthmatic response
ELISA	Enzyme-linked immunosorbent assay
eSNP	SNP associated with gene expression levels
FDR	False discovery rate
feNO	Fraction of exhaled Nitric Oxide
FEV_1	Forced Expiratory Volume in 1 sec
gp130	Glycoprotein 130
GWAS	Genome-wide Association Study
HDM	House dust mite
HREC	Human research ethics committee
ICS	Inhaled corticosteroid
IL	Interleukin
IL-6R	Interleukin-6 receptor
IQR	Interquartile range
LAR	Late asthmatic response
LCL	Lymphoblastoid cell line
mIL-6R	Membrane-bound interleukin-6 receptor
OCS	Oral corticosteroid
OR	Odds ratio
PBMC	Peripheral blood mononuclear cell
PBS	Pharmaceutical Benefits Scheme
PC_{20}	Provocative concentration causing a 20% decrease in lung function
RA	Rheumatoid arthritis
SABA	Short-acting beta agonist
SAE	Serious adverse event
sIL-6R	Soluble interleukin-6 receptor
SNP	Single-nucleotide polymorphism
STAT	Signal transducer and activator of transcription
STOML2	Stomatin-like 2 protein
TCZ	Tocilizumab
TIO	Tiotropium

Introduction

INTRODUCTION

Asthma is a chronic disease of the airways that affects about 235 million people worldwide[2] and over 2.5 million people in Australia.[3] It is characterized by enhanced bronchial reactivity, airway inflammation and reversible airflow obstruction.[4] Briefly, when exposed to environmental stimuli such as allergens or cigarette smoke, the airways of asthmatics tighten and get inflamed,[5] reducing the air that reaches the lungs. This leads to the most common symptoms of asthma, which include wheeze, cough, shortness of breath and chest tightness.[5] These symptoms can be life-threatening and long-lasting, representing a health burden that significantly decreases the quality of life of asthmatics.

Yet, despite its significant burden, asthma remains prevalent and without cure. Current treatments provide relief to some patients, but have several limitations. Short- and long-term medications are the two major classes of existing treatments to relieve and control asthma.[4] Short-term medications are widely used, regardless of disease severity, and act fast on acute symptoms. For instance, short-acting bronchodilators re-open tightened airways and allow air to flow. However, despite providing symptom relief, short-term medications fail to treat ongoing airway inflammation. Conversely, long-term medications attenuate inflammation, hence being able to control persistent asthma and prevent exacerbations. For example, inhaled corticosteroids (ICS), a first-line treatment for patients with persistent asthma, act by simultaneously activating and suppressing the expression of several genes involved in inflammatory responses, and reduce the number of inflammatory cells in the airways.[6] Yet, these too have limitations: they are not effective for all asthmatics; they need to be taken regularly, which can result in long-term adverse effects such as osteoporosis or suppression of immune responses against infections;[7,8] and their effectiveness partly relies on patient adherence to treatment,[9-11] which is often inadequate.[11-13]

The need to develop improved treatments for asthma is clear. However, two major challenges need to be addressed: (1) the heterogeneous nature of the disease, and (2) the low success rate in drug development. Asthma heterogeneity is well recognised[14-16] – subtypes that share similar phenotypes are underpinned by different molecular mechanisms, and do not respond to the same therapies. Importantly, there is an unmet need to develop new treatments for poorly-controlled asthma subtypes, which do not respond to current medicines and, despite being the minority, represent the largest healthcare and economic burdens.[17] Monoclonal antibodies (mAbs) are a promising class of drugs to address the individual needs of different asthma subtypes. Unlike conventional treatments, mAbs target key inflammatory components. For example, omalizumab – the first mAb to be approved for

asthma treatment – targets immunoglobulin E (IgE), a key player in allergic asthma, which was one of the first recognized asthma subtypes.[18] MAbs against interleukin-5 (IL-5) have also been recently introduced into clinical practice to treat eosinophilic asthma subtypes,[19] and other mAbs, like anti-TSLP,[20] are currently in drug development to target other cytokines involved in the initiation and persistence of airway inflammation.[21] These examples illustrate both the importance of (1) better characterising asthma subtypes, and (2) developing personalised treatments for those subtypes. However, as previously mentioned, a major challenge in drug development is the low attrition rate.[22] Most drugs that reached human clinical trials in the last decade have failed to show adequate efficacy in phase II and III studies.[23] In asthma, this may partly be addressed by identifying drug candidates that are more appropriate for specific disease subtypes.

An effective way to narrow down drug candidates is through genetic studies. Specifically, genome-wide association studies (GWAS) identify genetic variants that are associated with disease risk at stringent significance thresholds, providing new leads for the study of underlying disease mechanisms. Importantly, GWAS have been estimated to pinpoint targets that are twice more likely to succeed in clinical drug development.[24] Thus, variants identified in these studies represent the most promising candidates to prioritise for functional studies and drug development.

Between 2007 and 2016, 39 asthma risk variants in low linkage disequilibrium (LD) with each other were identified through GWAS.[25] Of these, rs4129267 is a promising finding,[1] supported by observational and functional studies. This variant is located in the interleukin-6 receptor (*IL6R*) gene and the rs4129267:T risk allele, which was estimated to increase asthma risk by 1·09-fold ($P = 2.4\times10^{-8}$),[1] has been shown to associate with low lung function, severe asthma,[26] and high serum levels of the soluble isoform of IL-6R (sIL-6R).[27] Interleukin-6 (IL-6) can bind to sIL-6R, as it would to the membrane-bound receptor (mIL6R); the IL-6-sIL-6R complex then signals through the membrane-bound glycoprotein gp130. While gp130 is ubiquitously expressed, mIL-6R is only present in certain cells.[28,29] Thus, cells that do not express mIL-6R can only be stimulated by IL-6 in the presence of sIL-6R, a process termed trans-signalling.[29,30] Remarkably, higher concentrations of sIL-6R have been found in the serum[31] and airways[32] of asthmatic patients, when compared to healthy individuals. In addition, airway sIL-6R levels have been reported to increase further in asthma exacerbations.[31] Thus, as sIL-6R widens the number of IL-6 target cells, these observations highlight the significance of the IL-6 pathway in asthma pathology, and suggest that blockade of the IL-6 pathway may represent a treatment option for asthma. Functional studies that tested this hypothesis in mouse models of asthma have shown that anti-IL-6R treatment is able to attenuate airway

inflammation.[32,33] Yet, it is unlikely that this approach will be effective for all asthma subtypes. For example, Ullah et al. showed that the anti-inflammatory effects IL-6R blockade was specific to models with elevated airway expression of IL-6 and sIL-6R.[33] Thus, when translating these findings to humans, it is important to identify the group of patients that will more likely benefit from such treatment. As previously noted, the rs4129267:T risk allele is associated with increased sIL-6R levels and severe asthma.[26,27] Accordingly, these may be features that characterise the asthma subtypes prone to respond to anti-IL-6R therapy. Further studies are required to test this hypothesis.

Lastly, the fact that IL-6R is the target of a drug approved to treat rheumatoid arthritis (tocilizumab [TCZ]) further supports the study of this pathway as a treatment option for asthma. Drug repositioning is an approach that has been shown to have increased chances of successful drug development.[34] Thus, given its well-known safety and pharmacokinetics, TCZ represents a promising candidate to repurpose for asthma treatment.

The overarching aim of this thesis was to understand the significance of the IL-6 pathway in asthma and to evaluate whether its blockade represents a promising new treatment option.

Chapter 1

The contribution of the IL-6 signalling pathway to asthma pathophysiology

CHAPTER 1. THE CONTRIBUTION OF THE IL-6 SIGNALLING PATHWAY TO ASTHMA PATHOPHYSIOLOGY

The goal of this chapter was to review the epidemiology and biology of asthma, as well as the evidence that IL-6 signalling contributes to disease pathophysiology. This was important to help plan the proposed research projects and adequately interpret their results.

Introduction

Interleukins are small signalling proteins from the cytokine family that play a central role in cell-to-cell communication. Particularly, interleukins were named after first being found to mediate communication between ("inter-") leukocytes ("-leukin"),[35], but they were later reported to promote communication in a wider group of cells. Once produced, these soluble molecules can be released from cells and act in a paracrine or autocrine fashion. They bind to their target cells through cognate receptors and trigger intracellular pathways that modulate the activity of the cell. In certain circumstances – such as inflammatory or immune responses – target cells are activated, differentiate and proliferate in response to these stimuli. Accordingly, it is crucial that this communication is finely regulated to avoid improper outcomes, such as cells being activated at the wrong time or by inappropriate stimuli. Often, dysregulation of interleukin signalling results in disease pathology, and for that reason interleukins have been the focus of a wide range of studies.

Interleukin-6 (IL-6) was first described as a stimulatory factor released by T cells to induce antibody production by B cells, known previously as B cell stimulating factor 2 (BSF-2).[36] However, given its multiple roles, BSF-2 was studied in parallel under other names such as interferon β-2 and hepatocyte stimulating factor.[37] It was not until their aminoacid sequence was analysed that it became clear that these proteins were the same, and so were subsequently all integrated in the interleukin family under the IL-6 name. Indeed, depending on the circumstances, IL-6 can play diverse roles, which sometimes can even be opposing. For instance, IL-6 is well-known for its pro-inflammatory activities, such as promoting differentiation of immune cells,[38,39] but in certain situations it also possesses anti-inflammatory properties[40,41]. The numerous ways in which IL-6 acts are the reason why this cytokine is critical for many diseases, particularly immune-related diseases, such as rheumatoid arthritis and Crohn's disease.

Asthma is yet another immune-related disease with mounting evidence of IL-6 involvement. Several studies support the causal role IL-6 plays in disease development, rather than merely acting as a

biomarker of the underlying inflammatory process. Genetic data also support a causal role for IL-6 dysregulation in asthma risk. Nonetheless, how exactly IL-6 contributes to asthma is still not fully understood; importantly, it is unclear whether blocking IL-6 signalling might prevent or treat asthma symptoms. In this review, I will outline the role played by IL-6 in the pathology of allergic asthma and discuss the possible outcomes of targeting this pathway for asthma treatment.

Asthma

Epidemiology

Asthma is a common inflammatory disease of the respiratory system. Asthma affects approximately 235 million people worldwide[2] and has a particularly high incidence in developed countries. Asthma is the cause of an estimated 250 000 deaths per year,[42] which occur mainly in under-developed countries. Compared with other chronic diseases, mortality rate is relatively low. However, the prevalence of asthma continues to rise globally[42] and the biggest burden associated with this disease is its morbidity. The symptoms of asthma are frequent and need to be controlled constantly and effectively. Symptoms worsen by night-time and with physical activity, affecting essential activities such as sleep, exercise, work and school. Moreover, asthma is often accompanied by co-morbidities such as rhinitis[43,44], which aggravate asthma's effect on daily life. Despite the significant disease-associated morbidity, asthma patients with poorly controlled symptoms are common and, as a consequence, the number of hospitalizations and health care costs associated with asthma exacerbations are high.[45,46] Thus, while no cure currently exists for asthma, the significant disease burden despite existing treatments justifies the development of new and improved therapeutic strategies. This can only be achieved through a better understanding of the disease pathophysiology.

Pathophysiology

Asthma is a complex disorder that can be broadly characterized by three features of the airways: hyperreactivity, inflammation and reversible obstruction.[4] Asthmatics have hyperreactive airways, and so experience recurrent acute reactions to a wide variety of stimuli. Different stimuli nonetheless trigger a broadly similar inflammatory response, which involves acute inflammation, which with time can lead to airway remodelling ("scarring"); contraction of airway smooth muscle; and an increase in mucous production. As a result of these inflammatory processes, the airway lumen narrows, the air that reaches the lungs diminishes and asthma symptoms develop, including cough, wheeze (a whistling sound mainly heard with exhalation), shortness of breath and chest tightness.[5] Nonetheless, despite its core features, asthma is a highly complex disease with variable onset, severity, genetic

make-up, pathophysiology and response to treatments. As such, classifying asthma into more homogeneous subtypes is required to study the disease in more detail.

Numerous classifications systems have been developed in an attempt to categorize the heterogeneous facets of asthma. One of the first classifications opposed extrinsic to intrinsic asthma[47] – now respectively known as allergic (or atopic) and non-allergic asthma. The first described a subtype of asthma with early onset and triggered by inhaled substances (allergens) like pollens and house dust mites. Typically, these asthmatics produce antibodies against allergens (atopy) and subsequently develop an immune response. Intrinsic asthma, on the other hand, is generally characterised by the development of symptoms late in life and in the absence of a particular environmental trigger. This dual categorization seemed convenient to work with, both in clinical and laboratory settings. However, mounting evidence gradually revealed a similar inflammatory profile behind extrinsic and intrinsic asthma: allergen-specific T_H2-type T cells that were initially believed to associate only with atopic asthma, as well as the cytokines they produced, were soon shown to participate in non-atopic asthma as well.[48] It became clear that in both subtypes, the balance between effector and regulatory T cells was compromised, contributing to initiation and maintenance of airway inflammation.[49] This explained why the power to predict response to treatment based on this classification was low.

Many other classification methods were developed since then, with the goal of achieving improved diagnosis and response to treatment.[50] Subtypes based on observable clinical characteristics are often preferred by practitioners to classify patients and assign treatment. However, when it comes to asthma, this approach leads to an inconsistent classification, with overlapping subtypes and low predictive power regarding response to treatment. On the other hand, cellular and molecular phenotypes have the potential to more adequately characterize the pathways underlying the disease on a given patient and are more likely to provide guidance in treatment development and assignment. Indeed, in a clustering analysis performed in three asthma cohorts, Halder et al. found that a symptom-led approach was effective to assign treatment to some, but not all patients.[51] Specifically, patients with discordance between symptoms and eosinophilic inflammation were the ones that usually fail to respond to conventional treatment regimens. Thus, measuring airway inflammation (both at the cellular and cytokine level) appears critical to predict treatment response with greater accuracy.

The Halder et al. analysis also showed the value of multivariate approaches to dissect the heterogeneous nature of asthma. Indeed, the utility of integrating multidimensional information has

been demonstrated in a number of studies. For example, using a range of clinical variables in an agglomerative cluster approach, Moore et al. identified five groups of patients that differed clinically, physiologically and in terms of inflammatory parameters.[52]

Thus, as previously proposed,[53] a combined characterization at a phenotypic, cellular and molecular level represents a more reliable way of defining and targeting different asthma subtypes.

Genetics

Familial and twin studies have long demonstrated that environmental risk factors alone are not sufficient for asthma to develop; instead, a genetic predisposition is also required, which is estimated to explain >50% of disease liability.[54-56] However, unlike monogenic diseases, where one gene alone is responsible for the development of the disease, asthma is a complex disorder associated with many genetic risk factors, each with a relatively small effect. Thus, finding these genetic variants that are associated with asthma risk is not straightforward. Nonetheless, the significance of the genetic basis of asthma has prompted much research to identify specific risk loci.

Two main approaches have been used to identify asthma risk variants: candidate-gene studies and genome-wide association studies (GWAS).[57] Candidate-gene studies test genetic variants from genes that are believed to be associated with the disease. When the presence of an allele is more common among cases than controls (or vice-versa), the genetic variant is said to be associated with disease risk. This approach is useful to confirm the association with previously suggested risk variants, particularly if their effect is small. However, it is limited by *a priori* knowledge of which variants to test. GWAS on the other hand, represent a proven hypothesis-free approach to find genetic variants associated with asthma.[58] In these studies, hundreds of thousands (sometimes millions) of variants across the genome are compared between cases and controls. As this approach is not focused on previously suggested regions or genes, it can identify associations with variants that are located in new risk loci, with the potential to provide novel insights into the molecular mechanisms underlying the disease.

The first asthma GWAS was published in 2007.[59] Thousands of variants were tested for association with childhood asthma and a new susceptibility locus on chromosome 17q21 was identified, containing the *ORMDL3* gene. Since then, the association of this locus with asthma has been replicated in several studies[1,60-63] and functional analyses suggest that the protein coded by *ORMDL3* plays a role in several cellular processes that likely contribute to the pathology of asthma.[64] Numerous

other GWAS in asthma were subsequently published, and SNP-based heritability, which represents the variance explained by all single nucleotide polymorphisms (SNPs) included in a GWAS,[65] was estimated to range between 7 - 14%.[25,66] Importantly, in the 10 years following the first asthma GWAS, 73 unique genetic variants were reported to associate with asthma risk at genome-wide significance level ($P < 5 \times 10^{-8}$) across 25 GWAS.[25] Of those, 31 variants, which were (1) in low linkage disequilibrium (LD) with each other ($r^2 < 0.05$) and (2) reported in GWAS of European ancestry, were estimated to explain 2.5% of the variation in asthma liability.[25]

Interestingly, many loci containing cytokine-coding genes and genes involved in the regulation of cytokines were identified, suggesting that dysregulation of cytokine signalling is critical for the development of asthma. In this review, I will focus on the role played by IL-6 in asthma and review studies that provide evidence for the pathogenesis of this cytokine.

The role of interleukin-6 and its receptor in asthma

IL-6 in asthma

IL-6 is a small signalling molecule that certain cells release to interact with neighbouring cells. It is mainly produced by immune cells such as T- and B-lymphocytes, macrophages, eosinophils and granulocytes, but it can also be produced by other cell types such as fibroblasts and bronchial epithelial cells.[67-69] Both its synthesis and release occur when the cell is harmed or exposed to stress by stimuli such as pro-inflammatory cytokines or allergens.[70-72] For a long time, IL-6 was seen as a general marker of inflammation, but growing evidence now suggests that instead, it plays a causal role in inflammatory diseases and in some cases, like rheumatoid arthritis, inhibition of this signalling pathway has therapeutic efficacy.[73-75]

The role of IL-6 signalling in chronic inflammatory diseases is extensively reviewed in the literature.[76-78] IL-6 is a key player in acute phase responses[79,80] and dysregulation of IL-6 signalling has been shown to underlie diseases like rheumatoid arthritis (RA),[81,82] Castleman's disease,[83,84] and Crohn's disease.[85,86] Several studies have reported elevated levels of IL-6 in circulation and in the inflammatory microenvironment of these diseases,[87-90] which explains the association between this cytokine and multiple disease features. For instance, in RA, IL-6 mediates local inflammation, which causes destruction of the joints,[81] and in Crohn's disease high IL 6 levels associated with the severity of endoscopical and histopathological findings.[86]

IL-6 promotes $CD4^+$ T cell differentiation, and dysregulation of the IL-6 pathway has been shown to affect the balance between T_H1/T_H2 cells and T_H17/Treg,[38,91] which are key players in chronic inflammatory diseases. IL-6 is also known to promote angiogenesis and increased vascular permeability, both of which are pathological features of diseases like asthma, RA and Crohn's disease.[92-95] Importantly, blockade of the IL-6 signalling pathway showed anti-inflammatory properties in several disease models,[32,33,96-100] which prompted the start of clinical trials to study the effects of anti-IL-6 therapy in humans. Remarkably, several studies demonstrated the safety, tolerability and efficacy of tocilizumab – a humanized monoclonal antibody that blocks IL 6R – in RA patients, and tocilizumab is now approved for the treatment of RA in over 90 countries.[78] Tocilizumab is also approved for the treatment of systemic juvenile idiopathic arthritis (JIA), and Castleman's disease, and represents a promising drug to reposition for the treatment of other inflammatory diseases.[78]

Asthma is among the diseases that may benefit from anti-IL-6 therapies like tocilizumab. In 1995, Yokoyama et al. not only showed that asthma patients had higher serum IL-6 levels than healthy controls, but also that there was a further increase in IL-6 levels after an asthma exacerbation.[101] High levels of IL-6 were also observed in the airways of asthmatics in a number of studies[75,102] and sputum levels of IL-6 have been shown to correlate negatively with FEV_1 (forced expiratory volume in one second)[102,103] and positively with the Asthma Control Questionnaire (ACQ) – a scoring system that assigns higher grades to poorer controlled asthma.[102] These findings implicated the presence of IL-6 in the inflammatory environment of asthma, but further studies showed that IL-6 is not merely a by-product of inflammation, but rather a cytokine that acts independently of inflammation.[75]

IL-6 actively contributes to asthma inflammation by interfering with the balance between effector and regulatory T cells. Specifically, IL-6 has been shown to suppress the activity of regulatory T cells[104] and to promote differentiation of T helper 2 (T_H2) cells over T_H1.[38,105] T_H2 cells have long been associated with allergic asthma.[106] These cells release pro-inflammatory cytokines (e.g. IL-4, IL-5 and IL-13) that contribute to asthma pathophysiology through several mechanisms, including B cell isotype switching and IgE secretion; recruitment and differentiation of eosinophils; increased bronchial hyperresponsiveness; and mucus production.

IL-6 drives IL-4 production in $CD4^+$ naive T cells, promoting their differentiation to T_H2 cells.[107] In addition, IL-6 modulates the inflammatory responses of differentiated T_H2 cells. For example, IL-6 produced by alveolar macrophages (AM) has been shown to promote $CD4^+$ T-cell IL-5 production

upon allergen stimulation.[69] Interestingly, compared to AM from non-asthmatic subjects, AM from patients with atopic asthma produce increased amounts of IL-6,[69] and in mouse models of asthma, blockade of IL-6 signalling (either through knockout or antibody-mediated neutralization) markedly decreased allergen-induced IL-5 production, which abrogated the recruitment of eosinophils and neutrophils into the airways.[108] Likewise, blockade of IL-6 signalling has been shown to deplete allergen-induced production of IL-13,[33] which associated with attenuated airway hyperresponsiveness and mucus production.

In addition, recent studies provided evidence of IL-6 involvement in the regulation of T_H17 cells,[78,109,110] a distinct type of effector T cells that contributes to the inflammatory responses in asthma. Thus, altogether, these studies support a contribution of IL-6 to core features of asthma and to symptom variability observed amongst patients.[102,111]

The IL-6 receptor: two forms that activate the IL-6 classic- or trans-signalling pathways

As a soluble molecule, IL-6 exerts its biological actions by binding to a receptor anchored to the membrane of its target cells. Specifically, the IL-6 receptor is a complex composed of two subunits: a ligand-binding component, IL-6R, and a signal-transducing unit, gp130.[29,30,112] When IL-6 binds to IL-6R, two molecules of gp130 are recruited to form the complex and the signal is transduced into the cell.[113] The subsequent steps of this signalling pathway are extensively characterised in the literature.[74,114] Briefly, once IL-6 binds to its receptor, homodimerization of the gp130 molecules leads to activation of two intracellular signalling pathways – the JAK/STAT (Janus Kinase/Signal Transducer and Activator of Transcription) and the Ras/MAPK (Mitogen-Activated Protein Kinase) pathways. Activation of these pathways causes the translocation of specific transcription factors to the nucleus, which regulate the expression of IL-6 responsive genes, such as those coding for acute phase proteins. Thus, understanding the regulation of the IL-6 receptor complex has been critical to characterise the signalling pathway of this cytokine.

In part, the study of the IL-6 receptor complex has clarified the roles played by the cytokine itself. First, while IL-6R is exclusive to the IL-6 receptor complex – binding to IL-6 only – the signal-transducing subunit gp130 is shared with receptor complexes of other cytokines of the IL-6 family, such as LIF (Leukaemia Inhibitory Factor), OSM (Oncostatin M) and CNTF (Ciliary Neurotrophic Factor),[115]. This explains the redundancy of some activities of IL-6 and other cytokines from the same family. Second, while IL-6R is selectively expressed by a restricted group of cells, such as lymphocytes and macrophages, gp130 is ubiquitously expressed.[28,29] Since IL-6 does not

directly bind to gp130, only cells with IL-6R on their membrane can be stimulated directly by IL-6, a process called classical signalling. However, IL-6 can also stimulate cells through a soluble version of IL-6R (sIL-6R) – a process known as the trans-signalling pathway. In this pathway, IL-6 binds to sIL-6R and in turn, this complex is recognised by gp130, which initiates the intracellular signalling cascades. Thus, in the presence of sIL-6R, a wide range of cell types that express gp130 can be activated by IL-6, which underlies some of the pleiotropic effects of this cytokine.

Unlike other soluble receptors, sIL-6R does not counter the role of its membrane-bound version. Instead, it works agonistically with mIL-6R and widens the range of cell types that IL-6 can target. This is particularly relevant to interpret the reports of high systemic and airway levels of sIL-6R observed in asthmatic patients.[31,32] These observations raise the hypothesis that the trans-signalling pathway may play a role in the pathogenesis of asthma. Indeed, that hypothesis has been experimentally demonstrated in animal studies. Doganci et al.[32] showed that sgp130-Fc, a fusion protein that inhibits trans- but not classic IL-6 signalling, significantly reduced eosinophilia and Th2 cytokine levels in bronchoalveolar lavage fluid (BALF) after OVA challenge. Similarly, Ullah et al.[33] found that both neutrophilia and eosinophilia were attenuated by sgp130-Fc, which was mirrored in significant decreases in IL-13, IL-17A and IL-17F levels in BALF.

The soluble version of IL-6R is thought to arise due to at least three mechanisms: (1) proteolytic shedding of the membrane bound form of IL-6R (mIL-6R),[116] which accounts for most variation in sIL-6R levels; (2) differential splicing of exon 9, which produces an isoform that directly encodes sIL-6R[117]; and (3) release of microvesicle-associated IL-6R.[118] Shedding of sIL-6R from the membrane of cells can be induced by various proteases like ADAM10 or PMA. Importantly, certain bacterial proteases have been shown to induce shedding of IL-6R, suggesting that certain types of allergens that contain bacteria may promote inflammation through a significant increase of the levels of sIL-6R in the airways. Indeed, in our own animal studies,[33] we found that two of the most common types of allergens – house dust mite (HDM) and cockroach – affect mIL-6R shedding differently. Specifically, while HDM allergen only increased IL-6 levels, cockroach increased both IL-6 and sIL-6R, suggesting that not all allergens contribute to an increase in IL-6 trans-signalling.

Undoubtedly, animal studies have greatly contributed to our understanding of the pathogenicity of the IL-6 pathway in asthma. Nonetheless, translating these findings to humans is not always straightforward. Thus, it would be interesting to replicate some of these experiments in human cell lines, to determine whether IL-6 trans-signalling is also triggered differently in response to different

stimuli in humans. Furthermore, determining exactly which stimuli present in airborne allergens are responsible for activating the IL-6 trans-signalling pathway could provide some clues as to how activation of this pathway might be prevented.

Genetic variants in the IL-6 receptor and the risk of asthma

Evidence for an association between variants in *IL6R* and asthma was first published in a GWAS meta-analysis, which included 57,800 individuals.[1] In that study, asthma risk was estimated to increase by 1.09-fold for each copy of the rs4129267:T allele, which has a frequency of 36% in Europeans. The SNP rs4129267 maps to an intronic region of the *IL6R* gene, and is also associated with variation in serum levels of sIL-6R.[27,119] Specifically, the rs4129267:T asthma risk allele is associated with increased sIL-6R levels, due to its effect on two separate mechanisms: first, it is associated with increased shedding of mIL-6R; second, it promotes the transcription of an *IL6R* isoform that directly encodes for sIL-6R. [119,120] The former association is thought to arise because rs4129267:T is in strong LD with rs2228145:C (LD $r^2 = 0.99$ in Europeans), which corresponds to the 358Ala amino-acid variant that is located within the main mIL-6R cleavage site[121] and that increases receptor shedding by ADAM proteases.[120] As a result, the asthma rs4129267:T predisposing allele is associated with increased sIL-6R serum levels, which in turn have been shown to be elevated in asthma patients.[31] In addition, rs4129267:T was subsequently found to be associated with poor lung function and severe asthma.[26] Hence, these results strongly suggested that *IL6R* was indeed the target gene underlying the association observed between rs4129267 and asthma risk in the Ferreira et al. GWAS.

Unlike for other asthma risk variants (e.g. rs3771166 in the *IL18R1* gene), the association between rs4129267 and asthma has not been replicated at genome-wide significance level ($P < 5 \times 10^{-8}$). However, strong associations, with consistent direction of effect (i.e. with rs4129267:T as the predisposing allele) have been reported independent samples, including the UK Biobank GWAS ($P = 5.8 \times 10^{-6}$).[25] In addition, we found that a second independent but less common SNP in *IL6R* (rs12083537, minor allele frequency of 19%) was also associated with the expression of *IL6R* and asthma risk (OR = 1.05 [122]). Together, these findings suggested that increased asthma risk was associated with increased IL-6 trans-signalling and decreased IL-6 classical signalling. However, it is important to recognise that many more genes are part of (or interact with) the IL-6 pathway. The extent to which such genes might also represent risk factors for asthma is largely unknown. This information is important, as it could provide new targets (other than IL-6R) to inhibit the IL-6 signalling pathway in asthma.

Targeting the IL-6 pathway as a treatment strategy for asthma

As summarised above, there is extensive evidence linking the IL-6 pathway and asthma, suggesting that its inhibition might be an effective new treatment for this disease. Thus, tocilizumab – the anti-IL-6R drug approved for RA treatment – is a good candidate to reposition for the treatment of asthma.

Tocilizumab (TCZ) binds to both the soluble and the membrane-bound forms of IL-6R, thereby inhibiting both the classical and trans-signalling pathways.

In RA, human genetic studies suggest that TCZ is effective in reducing disease symptoms because it inhibits IL-6 classical signalling. This is because the rs4129267:T allele that associates with inhibition of IL-6 classical signalling (while it promotes trans-signalling), reduces the risk of developing RA.[123]

In asthma, unlike for RA, human genetic studies show that the rs4129267:T increases disease risk.[1] Because rs4129267:T not only reduces mIL-6R but simultaneously also increases sIL-6R, this protective effect on disease risk can be explained by either reduced IL-6 classic signalling or, alternatively, increased IL-6 trans-signalling. It is critical to determine which of these two alternatives is correct, because they imply opposing clinical effects of TCZ in asthma patients. Specifically, if rs4129267:T increases asthma risk because it reduces classic signalling, then TCZ would be expected to worsen asthma symptoms. Conversely, if rs4129267:T increases asthma risk because it increases trans-signalling, TCZ would be expected to improve asthma symptoms.

Experiments with animal models,[32] including our own,[33] suggest that the latter alternative is true. Anti-IL-6R drugs attenuated airway inflammation, suggesting that increased trans-signalling (rather than reduced classical signalling) is a risk factor for asthma. Therefore, although there is convincing evidence that TCZ is a promising new drug for asthma, clinical trials are now required to formally test this possibility.

Conclusions

Until recently, asthma management strategies have broadly consisted of three approaches: (1) prevention of disease onset and/or symptoms by avoiding/minimizing the exposure to recognised environmental risk factors; (2) prevention of symptoms with corticosteroids; and (3) symptom relieve with bronchodilators. However, given the complexity and heterogeneity of asthma, these are wide-ranging measures to which many individuals do not respond. A more efficient approach to manage this disease is to break it into more homogeneous subgroups, characterised based on the

underlying cellular and molecular components. To this end, the support of genetic studies that can identify new asthma risk genes is essential. Such genes point to pathways that are causally related to the inflammatory mechanisms underlying asthma. Targeting components of these pathways directly may prove particularly effective for asthma treatment.

Growing evidence supports the involvement of IL-6 in asthma, including that of genetic studies. Importantly, an association has been reported between sIL-6R and several asthma features. Given that this soluble version of the receptor is the only mechanism through which IL-6 is able to stimulate a wide range of relevant cell types, it is possible that the high concentrations of sIL-6R observed in asthmatics reflect an underlying genetically-caused dysregulation of gene expression that leads to disease. Thus, it is important to comprehensively characterise asthma patients with high IL-6 trans-signalling. Importantly, a multivariate approach combining information of clinical features (which may overlap with those of distinct groups of asthma) and biomarkers of this asthma subtype could give new insights into a specific type of asthma that could be targeted by anti-IL-6R drugs, such as tocilizumab.

It is important to also acknowledge that asthma is a complex disease at several levels, from phenotype down to genotype. Therefore, it is essential to integrate information from the several components that, together, contribute to the disease state. For instance, at the molecular level, regardless of our understanding of how the IL-6 pathway contributes to disease development, it is worth noting that this pathway does not act on its own. It is linked to other pathways that simultaneously contribute to disease pathophysiology, and which may explain other important clinical features. Thus, exploring those pathways is equally important, as it may provide further insight to the networks underlying disease risk.

Lastly, additional studies are needed to determine whether treatments such as tocilizumab may be used to treat asthma. So far, evidence suggests that this drug is a promising candidate to treat a subtype of asthma that involves activation of the IL-6 trans-signalling pathway. Thus, characterizing such a group of asthmatics, as well as testing whether they respond to such a treatment, is warranted.

Chapter 2

Clinical features of patients with a microenvironment in the airways that promotes IL-6 trans-signalling

CHAPTER 2. CLINICAL FEATURES OF PATIENTS WITH A MICROENVIRONMENT IN THE AIRWAYS THAT PROMOTES IL-6 TRANS-SIGNALLING

The goal of this chapter was to identify clinical subtypes of asthma with an underlying pathophysiology that is likely to involve the activation of the IL-6 trans-signalling pathway. Results from these analyses were included in a manuscript published in the Journal of Allergy and Clinical Immunology:

Ullah, M. A., **J. A. Revez**, Z. Loh, J. Simpson, V. Zhang, L. Bain, A. Varelias, S. Rose-John, A. Blumenthal, M. J. Smyth, G. R. Hill, M. B. Sukkar, M. A. Ferreira and S. Phipps (2015). "Allergen-induced IL-6 trans-signaling activates gammadelta T cells to promote type 2 and type 17 airway inflammation." J Allergy Clin Immunol 136(4): 1065-1073.

Introduction

The pathophysiology of asthma is not homogenous across all patients. Several attempts have been made to classify and discriminate distinct clinical phenotypes to help diagnosis and treatment.[53] For instance, asthma can be characterized based on the proportion of inflammatory cell types present in the lung. Relying exclusively on the proportion of sputum eosinophils and neutrophils, four distinct phenotypes can be distinguished:[124] eosinophilic (characterized by a high proportion of eosinophils), neutrophilic (high proportion of neutrophils), mixed granulocytic (high proportion of both cell types) and paucigranulocytic (low proportion of both cell types). These phenotypes reflect the type of ongoing inflammation and this information may be used to assign targeted treatments.[125] However, the mechanisms behind the different granulocytic infiltrations are not well understood but could be informative to develop novel asthma treatments.

Recent experiments with a mouse model of asthma have shown that allergic immune responses in the airways that are associated with high airway expression of IL-6 and sIL-6R – which promotes IL-6 trans-signalling – can be attenuated by IL-6R blockade.[33] Specifically, an allergen-induced inflammatory response characterized by mixed granulocytic infiltration and high IL-6 and sIL-6R levels in the airways was shown to respond to treatment with IL-6R inhibitors. Yet, it remained unclear if those findings extended to humans. It is possible that asthmatics with airway IL-6 trans-signalling define a clinically distinct group of asthma, exhibiting clinical features that either mediate or result from the presence of high levels of IL-6 and sIL-6R in the airways. Here, to

investigate whether IL-6 trans-signalling underlies a clinically distinct group of asthma, we phenotypically characterized asthmatics with high airway levels of IL-6 and sIL-6R.

Methods

We measured IL-6 and sIL-6R levels in induced sputum samples of asthma patients, and tested these for association with clinical phenotypes.

Study Participants

A total of 158 subjects with a doctor diagnosis of asthma were recruited from the greater Brisbane area between October 2012 and December 2013 and completed the clinical protocol. The participants were non-smokers, aged 12-60, and had no respiratory infection in the week preceding clinical testing. Clinical testing included completing a detailed questionnaire about asthma history, symptoms, severity, medication and triggers; skin prick testing against six common allergens; spirometry (Micromedical Microlab Mk 8); measurement of fractional exhaled nitric oxide (feNO, NIOX MINO); sputum induction; and blood collection. Participants were asked to withhold anti-histamines for 72 hours, inhaled steroids for 48 hours, non-steroidal preventers or symptom controllers for 24 hours and reliever medication for 8 hours before testing. For the present study, only individuals who reported still suffering from asthma were considered for analysis (n = 138). This study was approved by the QIMR Berghofer Human Research Ethics Committee and all subjects gave written informed consent.

Sputum induction and analysis

Sputum induction and processing were performed as described previously.[126] Briefly, a saline solution was inhaled for doubling periods (30 sec, 1 min, 2 min, and 4 min) from a DeVilbiss Ultrasonic nebulizer (UltraNeb) connected to a Hans Rudolph 2700 two-way valve box with rubber mouthpiece and nose clips. Forced expiratory volume in 1 sec (FEV_1) was measured 60 sec after each saline dose, after which participants were asked to expectorate into a sterile container. The test was stopped when either the FEV_1 had fallen by more than 20% or 15.5 cumulative minutes nebulisation time had elapsed. Sputum plugs were selected, dispersed with dithiothreitol (DTT) for a minimum of 30 minutes (maximum 70 min) at room temperature and filtered through a 60 μm nylon filter. A total cell count was then performed with a light microscope. The sample was then centrifuged at 400 g for 10 minutes; the supernatant was stored at -80 ºC and cytospin slides prepared and stained with May Grunwald and Giemsa. A differential cell count of 400 non-squamous cells was then performed.

Based on the differential cell count, the sputum inflammatory subtype was classified into four groups, as proposed previously[124]: eosinophilic (≥ 1% eosinophils), neutrophilic (≥ 61% neutrophils), mixed granulocytic (≥ 1% eosinophils and ≥ 61% neutrophils) and paucigranulocytic (< 1% and < 61% neutrophils).

Measurement of IL-6 and sIL-6R levels

Of the 138 current asthmatics tested, 68 provided an induced sputum sample. Of these, we excluded from analysis 35 sputum samples with high saliva contamination (> 40% squamous cells) or low cell viability (< 40%), thus resulting in 33 samples available for analysis. A 10-ml serum sample was collected from 32 of these 33 individuals immediately after sputum induction. The sample was centrifuged at 805 g for 10 min, and the serum layer extracted and stored at -80 ºC until analysis. ELISA assays (R&D Systems, Minneapolis, MN, USA) were used to measure IL-6 and sIL-6R levels according to the manufacturer's procedures. The optical density was determined using a BioTek PowerWave XS2 microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) at both 450 and 540 nm wavelengths. Results and standard curves were acquired with BioTek Gen5 2.0 Data Analysis Software.

The sIL-6R ELISA kit (catalog number DR600) is insensitive to IL-6, i.e. sIL-6R levels measured with this assay reflect the total amount of sIL-6R (free or bound to IL-6). The IL-6 kit (catalog number HS600B) is affected by concentration of sIL-6R is ≥ 20 ng/ml.

Effect of sputum treatment with DTT on IL-6 and sIL-6R levels

To determine if treatment of sputum samples with DTT might have affected the ELISA assays used, we first treated the assay standards for IL-6 with DTT and used these as test samples in the ELISA assay as done for the sputum samples. We found that the concentration of IL-6 in DTT-treated standards was severely underestimated (Figure 2.1 A), as previously reported.[127] Similar results were obtained with the sIL-6R assay (Figure 2.1 B).

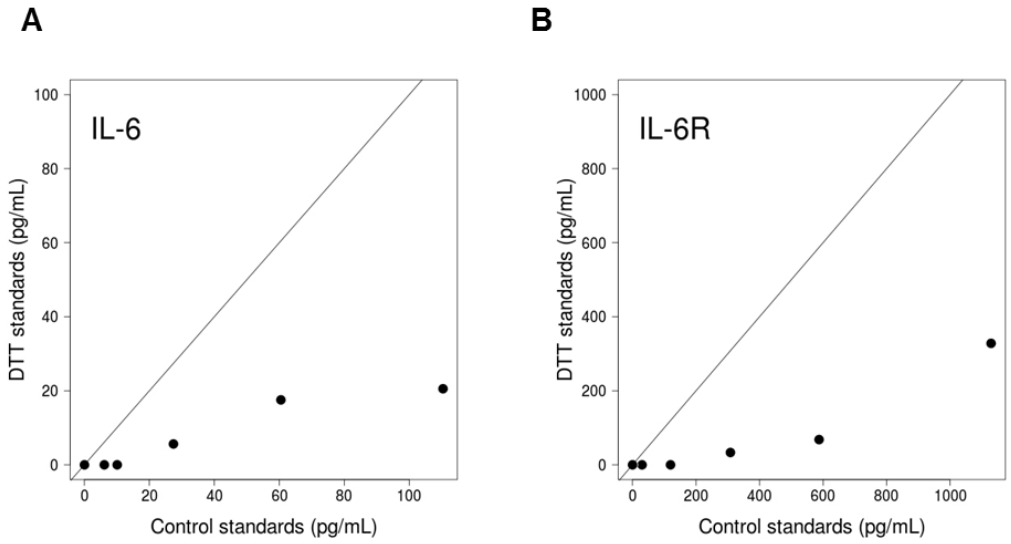


Figure 2.1. Correlation between the estimated concentration of assay standards treated with dithiothreitol (DTT, y-axis) and control standards (x-axis).

To prepare the DTT-treated standards (n = 6), the lyophilised assay standard included in the kit was first reconstituted at a higher concentration to allow for the subsequent addition of four volumes of 0.1% DTT to 1 volume of assay standard (4:1). Doubling dilutions were mixed, and the 0.1% DTT was added to each dilution. For comparison, a set of standards with the same concentration was prepared without DTT. Diagonal line indicates equality between the estimated concentrations of the two sets of standards.

Despite this, median IL-6 levels measured in sputum with the ELISA assay used in our study have been shown to be comparable between DTT-treated and DTT-untreated samples, suggesting that the activity of DTT is neutralized during the process of mucolysis, thus reducing its effect on the IL-6 immunoassay.[127] Furthermore, we found that if we used the standard curve obtained from the DTT-treated assay standards to determine IL-6 and sIL-6R concentrations in sputum samples, the resulting median sputum concentration levels (213 pg/ml and 788 pg/ml, respectively) were considerably higher than those reported in the literature for sputum or bronchoalveolar lavage fluid of asthmatics,[32,128-131] suggesting that they were overestimated. Collectively, these results are consistent with a significant effect of DTT on the detection of the assay standards but not (or less so) on the detection of the mediators in sputum samples. Therefore, we analysed IL-6 and sIL-6R sputum levels based on the standard curve obtained from the DTT-untreated assay standards.

Statistical analyses

Two sets of analyses were performed to assess the association between asthma clinical features and sputum levels of IL-6 and sIL-6R. First, we analysed sputum IL-6 and sIL-6R levels individually for association with clinical information. Second, we classified patients into two groups: those with high (i.e. above the median) IL-6 and sIL-6R (pro–IL-6–trans-signalling phenotype), and those with low IL-6 and/or sIL-6R sputum levels. Clinical features were then compared between these groups.

The association between sputum IL-6 and sIL-6R levels and clinical features of asthma was tested using linear regression, after normalising both dependent and independent variables with a non-parametric transformation. There were no significant effects of age, BMI or sex on IL-6 or sIL-6R levels. The association between the pro–IL-6–trans-signalling phenotype and clinical features was tested using a logistic regression. Results were considered significant at a *P*-value threshold of 0.05.

Power calculations were performed with the pwr R package to determine the effect size that our sample was able to detect with 80% power at a 0.05 significance level.

Analyses were performed with R version 3.2.3.

Results

To understand whether high airway levels of IL-6 and sIL-6R define a clinically distinct group of asthmatic patients – a potential target group for clinical trials of IL-6 inhibitors – we measured both IL-6 and sIL-6R levels in induced sputum samples of 33 patients with mild-to-moderate asthma (Table 2.1) and correlated this information with sputum immune cell counts and relevant clinical parameters. This sample was powered to detect effect sizes of 0.25 (i.e. $r^2 = 0.2$, or $r = 45$) with 80% power at a 0.05 significance level.

Sputum levels of IL-6 and sIL-6R were weakly correlated with each other (Figure 2.2 A), and largely independent of levels measured in matched serum samples (Figure 2.3), suggesting that they are determined by local (and not systemic) inflammatory responses.

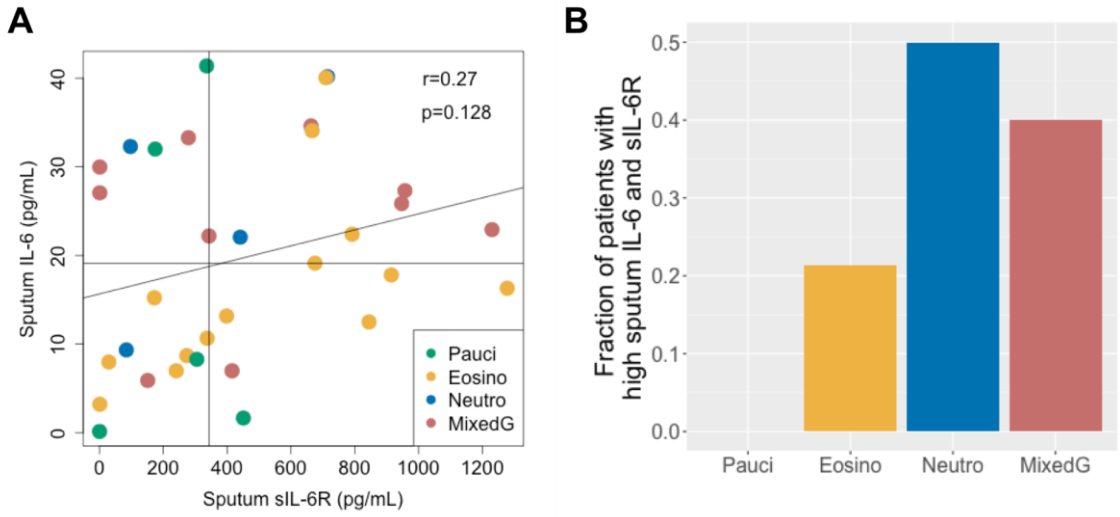


Figure 2.2. Correlation between IL-6 and sIL-6R levels in induced sputum samples from 33 mild to moderate asthmatics (A) and proportion of individuals with both high (above median) sputum IL-6 and high sputum sIL-6R (B).

Circle colours indicate sputum inflammatory subtype while vertical and horizontal black lines show overall median sIL-6R and IL-6 levels, respectively. Abbreviations: Eosino, eosinophililic; MixedG, mixed granulocytic; Neutro, neutrophilic; Pauci, paucigranulocytic.

	Eosinophilic	Mixed granulocytic	Neutrophilic	Paucigranulocytic
N	14	10	4	5
Female %	50	30	75	60
Mean age (range)	39 (21-59)	45 (21-58)	39 (29-52)	38 (31-45)
Median sputum total cell count, $x10^6$ / ml (IQR)	1.2 (0.9-1.5)	1.1 (0.8-1.5)	0.6 (0.4-0.9)	0.7 (0.6-0.8)
Mean sputum cell viability (range)	0.69 (0.42-0.93)	0.69 (0.54-0.82)	0.74 (0.68-0.84)	0.66 (0.58-0.82)
Mean differential cell counts, % (range)				
Squamous epithelial cells	10 (0-32)	10 (1-28)	6 (2-9)	16 (9-22)
Columnar epithelial cells	0.2 (0-0.7)	0.2 (0-1)	0 (0-0)	0.1 (0-0.7)
Neutrophils	42.4 (26-60)	78.5 (68-95)	76.7 (64-90)	33.1 (7-59)
Eosinophils	12.2 (1-35)	2.8 (1-7)	0.3 (0-1)	0.5 (0-1)
Macrophages	41.3 (12-65)	15.6 (4-27)	19.6 (8-30)	61.8 (39-89)
Lymphocytes	3.9 (0.7-21)	2.8 (0.2-5)	3.4 (1-5)	4.5 (2-7)
Mean absolute cell counts, $x10^4$ / ml (range)				
Neutrophils	57.9 (19.2-116.4)	112.7 (46.4-267.6)	61.6 (31.7-137.2)	29 (4.5-74)
Eosinophils	15.2 (1.3-40.5)	5.2 (0.9-26)	0.3 (0-0.5)	0.5 (0-1.2)
Macrophages	54.8 (12.9-114.8)	23.1 (3.1-56.4)	12.5 (8.6-20.2)	47.2 (24.7-61.9)
Lymphocytes	4.1 (0.8-18.1)	4.6 (0.2-14)	2.1 (0.7-3.6)	3.3 (2.1-4.7)
Mean baseline FEV_1 (range)	2.8 (1.2-4.8)	2.8 (0.8-5.4)	2.7 (1.8-3.3)	2.9 (1.8-3.5)
Mean percent predicted FEV_1 (range)	75.7 (43.9-94.4)	69.6 (34.2-113.2)	81.5 (65.9-107.9)	79 (56.7-98.1)
Mean FEV_1/FVC ratio (range)	0.71 (0.43-0.93)	0.64 (0.45-0.79)	0.74 (0.6-0.86)	0.72 (0.62-0.84)
Mean Asthma Control Questionnaire score (range)	1.73 (0.71-2.86)	1.67 (0.71-3.14)	1.96 (1.14-3.57)	1.8 (0.71-2.71)
Median FeNO (IQR)	34 (29-80)	44 (24-81)	12 (9-16)	27 (22-49)
Asthma severity (N in GINA steps 1:2:3:4:5)	6:0:6:2:0	2:1:5:1:1	1:0:1:2:0	2:0:0:3:0
Median sputum IL-6, pg / ml (IQR)	14.2 (9.2-18.8)	26.5 (22.4-29.3)	27.2 (18.9-34.3)	8.3 (1.7-32.0)
Median sputum sIL-6R, pg / ml (IQR)	532 (249-771)	379 (183-876)	269 (94-510)	305 (175-336)

Table 2.1. Clinical and sputum characteristics of 33 mild-to-moderate asthmatics who participated in this study.

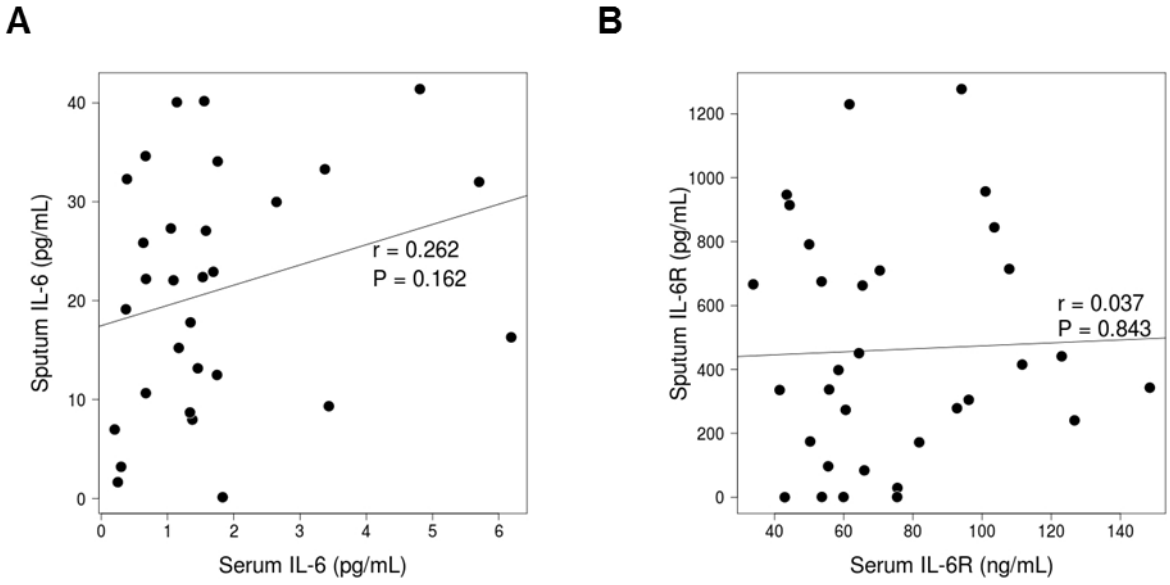


Figure 2.3. Correlation between sputum IL-6 and serum IL-6 (A) and between sputum sIL-6R and serum sIL-6R (B) in 33 mild-to-moderate asthmatics.

Consistent with this hypothesis, there were significant positive correlations between the total number of immune cells present in induced sputum and both sIL-6R and IL-6 levels (Figure 2.4). When the four major immune cell types present in sputum were analysed individually, we found significant correlations between sIL-6R levels and absolute numbers of both neutrophils and eosinophils and between IL-6 levels and neutrophil numbers (Table 2.2).

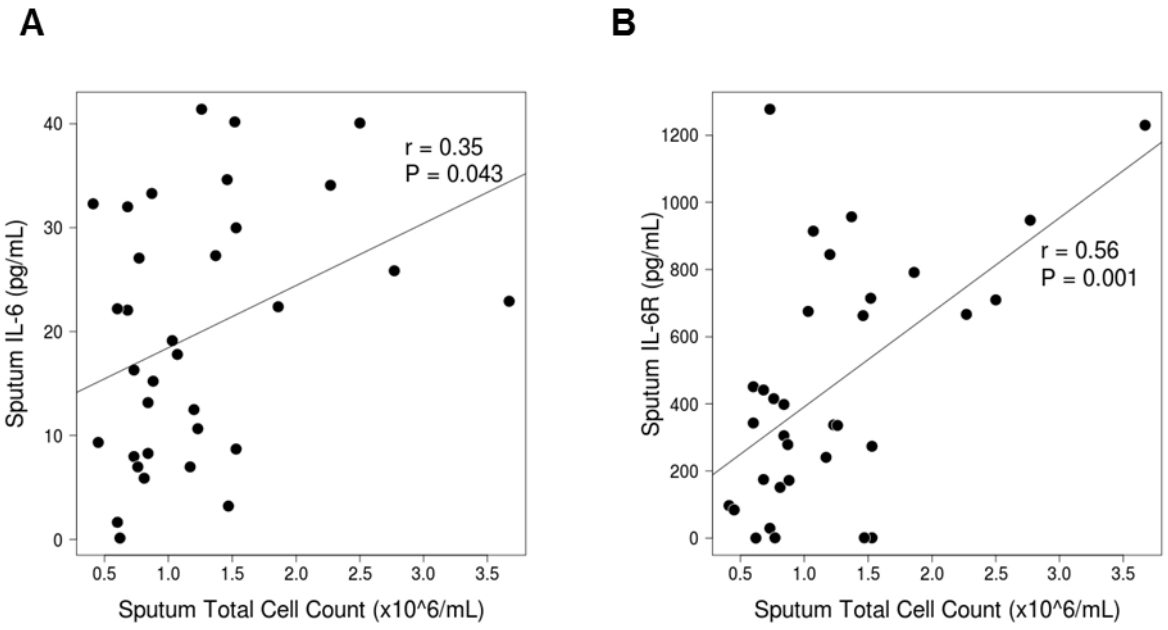


Figure 2.4. Correlation between sputum total cell count and sputum levels of IL-6 (A) and sIL-6R (B) in 33 mild-to-moderate asthmatics.

Outcome	Predictor	Scale	β	SE	*P*-value
sIL-6R	Neutrophils	Absolute, x 10^4 / ml	0.414	0.163	0.0165
		Relative, %	0.109	0.179	0.5460
	Eosinophils	Absolute, x 10^4 / ml	0.454	0.160	0.0080
		Relative, %	0.405	0.164	0.0193
	Lymphocytes	Absolute, x 10^4 / ml	0.111	0.178	0.5387
		Relative, %	-0.115	0.178	0.5240
	Macrophages	Absolute, x 10^4 / ml	0.223	0.175	0.2116
		Relative, %	-0.130	0.178	0.4700
IL-6	Neutrophils	Absolute, x 10^4 / ml	0.540	0.151	0.0012
		Relative, %	0.423	0.163	0.0142
	Eosinophils	Absolute, x 10^4 / ml	0.124	0.178	0.4924
		Relative, %	0.070	0.179	0.6990
	Lymphocytes	Absolute, x 10^4 / ml	-0.152	0.178	0.3998
		Relative, %	-0.282	0.172	0.1123
	Macrophages	Absolute, x 10^4 / ml	0.007	0.180	0.9706
		Relative, %	-0.324	0.17	0.0657

Table 2.2. Association between normalised sputum IL-6 and sIL-6R levels and sputum immune cell counts from 33 mild-to-moderate asthmatics.

To estimate the association between an IL-6 trans-signalling–promoting environment in the airways and the sputum inflammatory subtype, we classified asthmatic patients into two groups, those with high (i.e., above the median) IL-6 and high sIL-6R levels (n = 9) and those with low (below the median) IL-6 levels, low sIL-6R levels, or both (n = 24). The pro–IL-6–trans-signalling phenotype was more common in patients with neutrophilic (50%) and mixed granulocytic (40%) asthma when compared with those with eosinophilic (21%) and paucigranulocytic (0%) asthma (Figure 2.2). Consistent with this, neutrophils counts per gram of sputum were significantly (P = 0.0072) elevated in the pro–IL-6–trans-signalling phenotype.

Asthma clinical outcomes were comparable between patients with and without the pro-IL-6-trans-signalling phenotype (Table 2.3). Among the 33 patients with mild-to-moderate asthma tested, four (12%) had mixed granulocytic infiltration associated with both high IL-6 and high sIL-6R levels in sputum (Figure 2.2).

Outcome	β	SE	*P*-value
FEV_1	0.376	0.390	0.3420
FEV_1 % predicted	0.489	0.386	0.2139
FEV_1/FVC ratio	0.303	0.392	0.4459
Asthma symptoms	-0.185	0.394	0.6422
FeNO	0.353	0.390	0.3724
Asthma severity	0.391	0.389	0.3231

Table 2.3. Association between normalised asthma clinical outcomes and the pro-IL-6-trans-signalling phenotype.

Discussion

To assess the clinical implications of IL-6 trans-signalling in asthma, we studied the association between clinical features of asthma and both sputum IL-6 and sIL-6R levels. Sputum levels of IL-6 and sIL-6R did not correlate with levels measured in matched serum samples. These results may have been confounded by IL-6 binding to sIL-6R. Specifically, the ELISA assay used to measure IL-6 is affected by high concentrations of sIL-6R ($\geq$ 20 ng/ml). Consequently, the elevated concentrations of sIL-6R observed in sera may have affected the quantification of IL-6 in those samples. Importantly, measurements performed in sputum samples, which reflect the inflammations of the airways, showed relatively low levels of sIL-6R. Thus, it is unlikely that our measurements of IL-6 in sputum were affected.

High levels of both IL-6 and sIL-6R were more common in asthmatic patients with a neutrophilic or mixed granulocytic sputum profile when compared with those with the eosinophilic or paucigranulocytic inflammatory subtypes. These results are in line with recent observations from Chu et al.,[108] who reported elevated IL-6 levels in sputum samples of patients with mixed granulocytic bronchitis, and suggest that an airway environment that promotes IL-6 trans-signalling might be a risk factor for asthma exacerbations associated with high numbers of infiltrating neutrophils. But the reverse could also be true: a neutrophil-rich immune response might promote IL-6 trans-signalling in the airways.

We also found that 12% of patients with mild-to-moderate asthma tested had a sputum inflammatory profile – mixed granulocytic infiltration with high IL-6 and high sIL-6R levels – similar to that seen to attenuate with IL-6 blockade in mice experiments.[33] In light of this, we suggest that this is the group of patients most likely to respond to treatment with IL-6R inhibitors.

Our study does, however, have three main caveats. First, sample size was small; sputum total cell count and cell viability were also lower than reported in previous studies that used the same protocol for sputum induction and analysis,[124,132,133] although this might reflect the milder asthma spectrum sampled in our study. Second, we did not study a population of patients with severe asthma, the most likely to benefit from novel directed therapies. Sputum IL-6 and sIL-6R patterns in patients with severe asthma might be distinct from those identified in this study. Lastly, measurements of sputum IL-6 and sIL-6R levels might have been affected by the use of dithiothreitol (DTT) during sample processing, although we note that (1) DTT treatment was shown previously to have no effect on IL-6 levels measured in sputum[127] and (2) the observed median value for sIL-6R was consistent with that reported in BALF of asthmatic patients.[32] In addition, all samples in our study are likely to have been underestimated as they were all processed with DTT. Thus, the patients that we identified to have the highest sputum levels of IL-6 and sIL-6R are likely to be the same that would have been identified if samples had not been processed with DTT.

Nevertheless, despite these limitations, our results are consistent with recent observations from a study that evaluated the association between epithelial IL-6 trans-signalling activation and molecular and clinical phenotypes in asthmatic patients.[134] In that study, human bronchial epithelial cells were first stimulated with IL-6 and sIL-6R to identify genes induced by IL-6 trans-signalling. Then, using the most strongly-induced genes, the authors performed cluster analysis in a cohort of 103 asthmatic patients to identified the subset of patients with signs of lung epithelial IL-6 trans-signalling activation (n =17). The patients with the IL-6 trans-signalling gene signature were shown to have a history of frequent exacerbations and significantly increased infiltration of T cells and macrophages to the airways compared to the other asthmatics. In addition, and consistent with our results, sputum eosinophilia was significantly higher in that group of patients, and there was a trend, although not significant, towards increased sputum neutrophil counts. Thus, while our results should be interpreted with caution, they are supported by these observations.

In conclusion, we demonstrate that, in human subjects, an airway microenvironment that promotes IL-6 trans-signalling is associated with mixed granulocytic asthma. The latter could develop, for example, because of a genetic predisposition and/or exposure to specific allergens that trigger a neutrophil-rich inflammatory response.

Chapter 3

Sputum cytology during late phase responses to inhalation challenge with different allergens

CHAPTER 3. SPUTUM CYTOLOGY DURING LATE PHASE RESPONSES TO INHALATION CHALLENGE WITH DIFFERENT ALLERGENS

In the previous chapter, high airway levels of IL-6 and sIL-6R were found to be more frequent in subjects with mixed granulocytic and eosinophilic sputum inflammatory subtypes. In this chapter, the goal was to assess whether sputum inflammatory subtypes can be influenced by common asthma triggers. Results from these analyses were published in the Allergy journal in 2018:

Revez JA, Killian KJ, O'Byrne PM, Boulet LP, Upham JW, Gauvreau GM, Ferreira MAR: Sputum cytology during late-phase responses to inhalation challenge with different allergens. Allergy 2018 (in press).

Introduction

Allergic asthma is triggered by the inhalation of aero-allergens in previously sensitized individuals. Within hours of allergen exposure, various immune cell types infiltrate the airways, where they release pro-inflammatory mediators that result in narrowing of the airways and the characteristic symptoms of wheezing and shortness of breath.[135] Amongst the most abundant cells in the airway immune infiltrate are eosinophils and neutrophils. The relative abundance of these cell types in induced sputum varies widely between asthmatics and is often used to categorize the underlying inflammatory response.[124,136-139] Currently, four major sputum subtypes have been described[124,140] (1) eosinophilic, with elevated eosinophils but not neutrophils; (2) neutrophilic, with elevated neutrophils but not eosinophils; (3) mixed granulocytic, with both eosinophils and neutrophils elevated; and (4) paucigranulocytic, with both eosinophils and neutrophils within the normal range. Recognising this heterogeneity in inflammatory subtype is important because it can help identify the subsets of patients that can benefit most from specific targeted therapies.[141] For example, patients with eosinophilic asthma typically respond well to corticosteroids,[142-144] which is not the case for those with non-eosinophilic asthma[144-147] However, despite its importance, the factors that contribute to the heterogeneity in sputum inflammatory subtype in humans remain poorly understood.

Mouse models of asthma have provided some clues into the factors that might contribute to the type of sputum inflammatory subtype that develops upon allergen challenge. As in humans, airway inflammation can be induced in mice by challenging previously sensitized animals with a specific allergen. The allergen most commonly used to trigger airway inflammation in mice is ovalbumin (OVA), which typically induces eosinophilic inflammation. However, higher concentrations of

endotoxin in the OVA solution can shift the inflammation developed in the airways to a mixed granulocytic subtype.[148,149] Furthermore, the adjuvant that is co-administered with OVA to stimulate the immune response also influences the inflammation that develops in the airways. For example, co-administering alum promotes stronger eosinophilia than if no adjuvant is used.[150] Similarly, house dust mite (HDM), a common household allergen, is frequently used in mouse models of asthma and triggers an airway inflammation rich in eosinophils.[151,152] In contrast, cockroach and *Alternaria* allergens, to which humans are also commonly sensitized, promote an inflammation rich in both eosinophils and neutrophils in mice.[33,153,154] Less common are allergens that induce an increase in neutrophils but not eosinophils in mice: 1,3-β-glucan, the major cell wall component of fungi, is one example.[155,156] Collectively, these observations suggest that different allergen types are capable of promoting distinct inflammatory subtypes in mice.

To our knowledge, no study has previously tested the hypothesis that different allergens can induce distinct inflammatory subtypes in humans. As in mice, the inflammatory response to allergen exposure can be studied in humans in a controlled setting using an allergen inhalation challenge test. In this test, subjects with mild allergic asthma inhale increasing concentrations of an allergen solution until an early asthmatic response (EAR) is triggered, typically consisting of a 20% drop in lung function from baseline.[157] The allergen used in the inhalation challenge test is usually that which triggers the largest allergic response in a skin prick test.[157] The primary outcome measured is often the late asthmatic response (LAR), which follows the natural recovery from the EAR and corresponds to the drop in lung function from baseline recorded between 3 and 8 h after allergen challenge. The magnitude of the LAR reflects the severity of airway inflammation induced by allergen exposure,[158] and can vary considerably between individuals. Importantly, Boulet et al. found that some of this variation in the magnitude of the LAR can be explained by the allergen type used in the challenge.[159] On the other hand, the allergen used in the inhalation challenge did not determine the absolute number of eosinophils or neutrophils measured in sputum, but these cell types were analysed individually and sputum inflammatory subtypes were not considered. Here, we extend the analysis of Boulet et al. to test the association between sputum inflammatory subtypes and the allergen type inhaled, based on information from 129 individuals with a documented EAR and LAR.

Methods

Study design and participants

We performed a retrospective analysis of data from 21 clinical trials of experimental asthma treatments that included an allergen inhalation challenge test. The trials (summarized in Table 3.1) were conducted between 2003 and 2013 in two centers (Laval University and McMaster University); protocols were standardized between sites. Briefly, in all studies, each participant completed an allergen inhalation challenge test during the screening phase of the trial, that is, prior to receiving any experimental treatment. The test was carried out on three consecutive days and consisted of: (1) skin prick titration test and methacholine challenge test on day one, to determine the dose of allergen to be administered in the inhalation test; (2) the allergen inhalation challenge test on day two; and (3) a follow-up methacholine challenge on day three. All measurements included in our analysis refer to data collected only in these three days. A total of 153 participants (57% female) were recruited, aged 18 - 61 years. Subjects had mild asthma, defined by (1) a Forced Expiratory Volume in 1 sec (FEV_1) > 70% of the predicted normal value (i.e. sufficient lung function such that a 50% fall in FEV_1 would be safe and tolerated), (2) a provocative concentration of methacholine that caused a 20% drop in FEV_1 (PC_{20}) that was ≤ 16 mg/ml (see below), and (3) no requirement for anti-inflammatory treatments that could interfere with allergen-induced responses, with the exception of short-acting beta agonists (SABA) to relieve symptoms less than twice weekly.

Skin-prick tests

Each participant completed two skin prick tests – an allergen skin test and an allergen skin titration test.

The aim of the allergen skin test, typically performed during the first clinical visit, was to determine the allergen type to be used subsequently in the inhalation challenge test. Briefly, skin reactivity was measured 15 minutes after exposure (scratching or pricking) to (1) an allergen; (2) a positive control (1 mg / ml histamine); and (3) a negative control (diluent). Allergens tested included alder, white birch, horse, *Alternaria*, *Dermatophagoides farinae* (HDMDF), ragweed, grass, cat and *Dermatophagoides pteronyssinus* (HDMDP), and were purchased from Omega Laboratories, Greer and ALK. Positive skin reactions were defined as a wheal (raised itchy bump) greater than 2 x 2 mm in size. The allergen that resulted in the largest positive skin reaction after exposure was selected for the inhalation challenge (the test allergen).

Study	N participants	Sex (% female)	Age (years)	BMI (kg/m^2)	Allergens tested (allergen type, N)
1	22	66.7	32 ± 11.2	25 ± 4	*Alternaria*, 1; Cat, 5; Grass, 3; HDMDF, 1; HDMDP, 6; Horse, 4; Ragweed, 2
2	20	50	33 ± 13.2	26 ± 4.6	Alder, 1; Cat, 8; Grass, 3; HDMDP, 6; Ragweed, 1; White birch, 1
3	11	54.5	26 ± 8.3	24 ± 2.1	Cat, 4; Grass, 1; HDMDF, 1; HDMDP, 2; Horse, 1; Ragweed, 2
4	24	45.8	26 ± 8.7	NA	*Alternaria*, 1; Cat, 6; Grass, 5; HDMDP, 9; Ragweed, 3
5	9	55.6	25 ± 9.8	NA	*Alternaria*, 1; Cat, 2; HDMDP, 5; Ragweed, 1
6	10	60	31 ± 12.6	NA	Cat, 4; Grass, 1; HDMDF, 1; HDMDP, 4
7	18	52.4	31 ± 13.7	25 ± 5.9	Alternaria, 2; Cat, 6; Grass, 3; HDMDF, 2; HDMDP, 7; Ragweed, 1
8	11	54.5	38 ± 13.2	28 ± 8.7	Alternaria, 1; Cat, 5; Grass, 1; HDMDP, 4
9	21	57.1	27 ± 10.6	NA	Cat, 4; Grass, 2; HDMDF, 3; HDMDP, 10; Ragweed, 2
10	10	30	33 ± 15.3	24 ± 2.7	Alternaria, 1; Cat, 2; HDMDF, 1; HDMDP, 4; Ragweed, 2
11	7	28.6	29 ± 11	NA	Alternaria, 1; Cat, 1; Grass, 1; HDMDP, 3; Ragweed, 1
12	21	42.9	29 ± 10.3	NA	Cat, 11; Grass, 2; HDMDF, 1; HDMDP, 7
13	5	80	32 ± 15.4	25 ± 4.8	Alternaria, 1; Cat, 1; Grass, 1; HDMDP, 2
14	20	52.4	36 ± 13.7	27 ± 6.3	Cat, 9; Grass, 2; HDMDP, 8; Ragweed, 2
15	15	46.7	29 ± 10.6	NA	Cat, 3; Grass, 5; HDMDF, 2; HDMDP, 5
16	11	72.7	29 ± 10.8	NA	Cat, 2; Grass, 2; HDMDF, 2; HDMDP, 5
17	10	30	33 ± 13.3	NA	Cat, 1; HDMDF, 1; HDMDP, 6; Ragweed, 2
18	19	73.7	27 ± 11	NA	Cat, 3; HDMDF, 2; HDMDP, 9; Ragweed, 5
19	13	64.3	31 ± 13.1	27 ± 5.8	Cat, 5; Grass, 2; HDMDP, 5; Horse, 1; Ragweed, 1
20	7	57.1	33 ± 14.9	NA	Cat, 2; Grass, 3; HDMDP, 2
21	16	75	26 ± 9.1	NA	Grass, 2; HDMDF, 3; HDMDP, 9; Ragweed, 2

Table 3.1. Demographics of participants from 21 clinical trials of experimental asthma.

Results are presented as means ± SD. Abbreviations: BMI, body mass index

The allergen skin titration test was performed the day before the inhalation challenge test. The aim of this test was to determine the weakest dilution of the test allergen that resulted in a positive (> 2 mm wheal) skin prick response. Briefly, skin reactivity was measured 10 minutes after exposure to serial dilutions of the test allergen. The lowest titration that caused a positive reaction corresponded to the 'skin test end point', which is required to calculate the PC of the test allergen that is expected to cause a 20% drop in FEV_1, that is, the allergen PC_{20}.[160]

Methacholine inhalation challenge

A methacholine challenge test was performed the day before the allergen inhalation test to quantify airway sensitivity, as this is required to calculate the expected allergen PC_{20}, in addition to the skin test end point.[160] Briefly, methacholine chloride stock was prepared at a concentration of 128 mg/ml in physiological saline and further diluted in physiological saline to achieve working concentrations from 0.031 mg/ml to 128 mg/ml. A Wright nebulizer with an output of 0.13 ml/min was used to produce methacholine aerosols. Participants were instructed to wear nose clips and to breathe normally from a Hans Rudolf valve connected to the nebulizer, during a 2-minute period. First, subjects inhaled normal saline, then doubling concentrations of methacholine for periods of 2 min. FEV_1 was measured at 30, 90, 180 and 300 seconds after each inhalation, until a fall of 20% in FEV_1 was observed. The concentration at which a 20% decline in FEV_1 was observed corresponds to the methacholine PC_{20}.

Allergen inhalation challenge

The allergen PC_{20} was determined based on the skin test endpoint and methacholine PC_{20} using an equation developed elsewhere.[160] In turn, the allergen PC_{20} determined the first concentration of the test allergen that was administered in the inhalation test, specifically corresponding to three to four doubling dilutions below the PC_{20}. Starting with that concentration, allergen was inhaled at tidal breathing for 2 minutes, and FEV_1 measured 10 minutes after challenge. Increasing doubling concentrations of allergen (up to 1:4) were administered until a concentration was reached that caused a 20% decline in FEV_1, which corresponds to the observed allergen PC_{20}. FEV_1 was then recorded at regular intervals until 7 hours after inhalation of the last allergen dose. At 7 hours all participants were treated with a SABA to reverse bronchoconstriction from allergen challenge. Participants received no anti-inflammatory medications, including inhaled corticosteroids, before or after allergen challenge, with the exception of SABA to relieve symptoms less than twice weekly.

A positive early asthmatic response (EAR) was defined by a decline of ≥ 20% in FEV_1 from baseline within 3 h of the last allergen inhalation. A positive LAR was defined by a decline of ≥ 15% in FEV_1 3 - 7 hours post allergen inhalation. Only individuals who experienced both an EAR and an LAR were selected for analysis.

Sputum collection and classification of inflammatory subtype

Sputum was collected at three time points: after the methacholine challenge on day one, after the allergen challenge on day two (7 hours post inhalation), and after the methacholine challenge on day three. Relative to the start of the allergen inhalation test, we refer to these time points as -24 h, 7 h and 24 h post-challenge. The sputum samples collected therefore reflect three different inflammatory states: baseline (i.e. pre-challenge), LAR and persistence of LAR.

Sputum was induced and processed as described previously,[161] which included obtaining total and differential cell counts. To account for differences in sputum yield, analyses of absolute cell numbers were performed relative to sputum weight. The proportion of eosinophils and neutrophils in sputum was calculated relative to the total cell count and used to categorize sputum inflammatory subtype into four categories as described elsewhere[124]: (1) eosinophilic, > 1.01% eosinophils and < 61% neutrophils; (2) neutrophilic, < 1.01% eosinophils and > 61% neutrophils; (3) mixed granulocytic, > 61% neutrophils and > 1.01% eosinophils; or (4) paucigranulocytic, < 61% neutrophils and < 1.01% eosinophils. These thresholds were determined based on the 95th percentiles observed in a cohort of healthy individuals. [124]

Statistical analyses

Outcome variables measured on a continuous scale (cell counts, EAR and LAR) were assessed for normality using the Shapiro-Wilkinson test. Neutrophil counts, eosinophil counts and LAR were not normally distributed and so were transformed with a rank-based inverse-normal transformation. A paired *t*-test was used to compare cell counts between pre-challenge (-24 h) and post-challenge (7 h and 24 h) time points. A one-way ANOVA was used to test the effect of allergen type on variation in outcome variables, with post-hoc comparisons between allergen types performed with the Tukey-Kramer test. Associations between sputum subtype, which is a categorical variable, and predictors of interest (e.g. allergen type) were tested with a multinomial log-linear model, using the multinom function from the nnet R package.[162] Sputum subtype proportions were compared between groups using the McNemar test. All outcomes were assessed for association with covariates (age, sex, height, weight, and body mass index) before analysis.

Power analyses were performed with the pwr and the pwr2 R packages. Analyses were performed using R version 3.2.3.

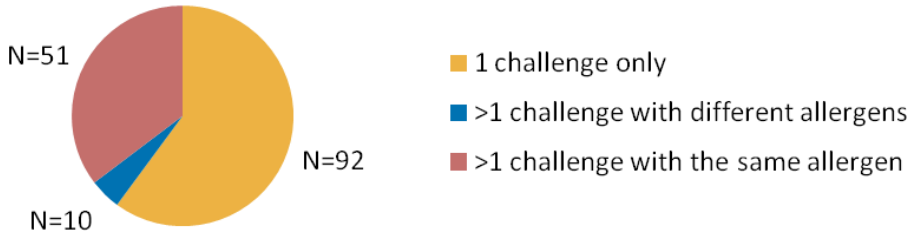
Results

Dataset and subject characteristics at baseline

The dataset available for analysis included 305 allergen challenges performed by 153 unique individuals and including nine allergen types (Figure 3.1).

A)

Number of subjects who performed allergen inhalation challenges:



Number of subjects challenged: 153
Total number of allergen challenges: 305

Number of allergen inhalation challenges performed with each allergen type:

	Alder	White birch	Horse	Alternaria	HDMDF	Ragweed	Grass	Cat	HDMDP
Original dataset	1	1	6	9	20	27	39	84	118
From unique individuals retained for analysis	0	0	0	0	12	16	22	39	40

B)

N subjects	153	(1) →	145	(2) →	145	(3) →	145	(4) → 129
N allergens	9		5		5		5	5
N challenges	305		288		158		145	129

1) Remove allergens used in <10 challenges
2) Average outcomes of subjects with >1 challenge with the same allergen
3) Restrict data to challenges with least common allergens for subjects with >1 challenge with different allergens
4) Restrict to subjects who experienced an EAR and an LAR

Figure 3.1. Summary of steps used to obtain the dataset analysed.

(A) The original dataset consisted of 305 allergen challenges performed by 153 unique individuals, including nine different types of allergen. Most individuals (n = 92) completed a single allergen

challenge. Of the 61 individuals who completed two or more allergen challenges, 51 were challenged with the same allergen, and 10 were challenged with different allergens.
(B) Four data management steps were applied to select individual allergen challenges for analysis. First, challenges performed with an allergen extract (alder, white birch, horse, Alternaria) tested in < 10 individuals were excluded. Second, results from challenges performed by the same individual with the same allergen type in different studies were averaged. Third, for individuals challenged with different allergen types in different studies, results for the least common allergen(s) were selected for analysis. Fourth, results from individuals who did not experience both an EAR and an LAR were excluded.

Four allergen extracts (alder, white birch, horse and *Alternaria*) were tested in a small number of individuals (n < 10), so these data were excluded from further analysis. Sixty-one individuals participated in two or more clinical trials and so completed multiple allergen challenge tests. For each of these individuals, outcomes were averaged across allergen challenge tests performed with the same allergen type in different studies. If an individual was challenged with different allergens in different studies, data was included for the challenge performed with the allergen type that was least common in the dataset, to maximize the number of challenges with each allergen included in the analyses. Lastly, data was excluded from 16 allergen challenges with a negative EAR and/or negative LAR, as it was not clear if allergen challenge triggered a typical acute asthmatic response. After these exclusions, data were available for 129 allergen challenges performed by 129 individuals and including five allergen types: HDMDF (n = 12), ragweed (n = 16), grass (n = 22), cat (n = 39) and HDMDP (n = 40). Sputum samples were available for 118 subjects before allergen challenge (-24 h), and for 123 (7 h) and 124 (24 h) subjects after allergen inhalation challenge. For 111 subjects, sputum samples were available across all three time points.

Subjects subsequently tested with different allergen types had similar characteristics at baseline, including, for example, comparable sputum subtype frequencies (Table 3.2). Age, height, weight and BMI, had no significant effect on variation in sputum subtype frequencies.

Overall effect of allergen challenge on sputum granulocyte counts and inflammatory subtype

At baseline (i.e. before allergen challenge), the sputum inflammatory subtype of most individuals was either paucigranulocytic (38%) or eosinophilic (43%) (Table 3.3), i.e. less than 20% of the individuals had a sputum subtype rich in neutrophils.

	HDMDF	Ragweed	Grass	Cat	HDMDP
n	12	16	22	39	40
Sex (% female)	41.7	50	22.7	68.4	65
Age (years)	27 ± 10.5 (19-52)	23 ± 5.9 (18-40)	29 ± 10.4 (18-48)	31 ± 13.8 (19-60)	29 ± 10.5 (19-54)
Height (cm)	174 ± 12 (155-192)	170 ± 9.9 (150-186)	176 ± 10.9 (155-195)	170 ± 8.7 (155-187)	169 ± 8.4 (153-188)
Weight (kg)	71 ± 6.1 (63-77)	73 ± 10.1 (59-83)	86 ± 21.3 (53-115)	75 ± 17.9 (52-113)	69 ± 13.5 (50-101)
BMI (kg/m^2)	23 ± 1.5 (21-24)	26 ± 2.1 (24-29)	27 ± 3.5 (22-32)	27 ± 6 (19-41)	24 ± 3.6 (19-32)
LAR (% fall in FEV_1)	28 ± 7.7 (15-41)	24 ± 9.2 (15-49)	24 ± 6.7 (17-42)	22 ± 6 (16-44)	26 ± 7.2 (15-44)
Baseline sputum characteristics					
TCC (x 10^6/g mucus)	2 ± 1.3 (0-4)	5 ± 5.9 (0-24)	3 ± 4.1 (0-19)	3 ± 2.2 (1-12)	4 ± 2.8 (1-13)
Eosinophils (x 10^6/g mucus)	16 ± 33.6 (0-110)	10 ± 16.8 (0-61)	4 ± 6.6 (0-26)	12 ± 20.8 (0-111)	16 ± 34.6 (0-172)
Neutrophils (x 10^6/g mucus)	111 ± 52.2 (32-191)	286 ± 573.9 (2-2255)	185 ± 337.6 (4-1518)	140 ± 195.5 (1-915)	179 ± 212.7 (4-1022)
Subtype (% Pauci, % Eos, % Neut, % MG)	10, 60, 30, 0	60, 13, 13, 13	45, 35, 10, 10	31, 56, 3, 11	41, 43, 8, 8

Table 3.2. Demographics and clinical characteristics of the study participants, stratified by allergen type inhaled.

Results are presented as frequencies (in %) or means ± SD (range). There were no statistically significant differences in baseline characteristics between the groups. Abbreviations: BMI, body mass index; Eos, eosinophililic; FEV_1, forced expiratory volume in 1 s; LAR, late airway response; MG, mixed granulocytic; Neut, neutrophilic; Pauci. paucigranulocytic; TCC, total cell counts.

		-24h	7h		24h		
	Subtype	Proportion	Proportion	$P^{§}$	Proportion	$P^{§}$	$P^{†}$
All allergens		*n* = 118	*n* = 123		*n* = 124		
	Paucigranulocytic	0.38	0.06	$2.5x10^{-10}$	0.04	$4.5x10^{-9}$	0.4795
	Eosinophilic	0.43	0.55	0.0253	0.76	$3.6x10^{-7}$	$1.0x10^{-4}$
	Neutrophilic	0.09	0.01	0.0016	0.02	0.0114	0.3173
	Mixed granulocytic	0.09	0.38	$3.3x10^{-7}$	0.19	0.0184	$1.0x10^{-4}$
HDMDP		*n* = 37	*n* = 38		*n* = 38		
	Paucigranulocytic	0.41	0.05	$3.0x10^{-4}$	0.05	$3.0x10^{-4}$	1
	Eosinophilic	0.43	0.53	0.4669	0.76	0.0047	0.0126
	Neutrophilic	0.08	0.00	0.0833	0.00	0.0833	NA
	Mixed granulocytic	0.08	0.42	0.0029	0.18	0.1573	0.0126
Cat		*n* = 36	*n* = 38		*n* = 38		
	Paucigranulocytic	0.31	0.05	0.0016	0.00	0.0016	0.1573
	Eosinophilic	0.56	0.66	0.285	0.84	0.0067	0.0348
	Neutrophilic	0.03	0.00	0.3173	0.00	0.3173	-
	Mixed granulocytic	0.11	0.29	0.0522	0.16	0.3173	0.0956
Grass		*n* = 20	*n* = 19		*n* = 22		
	Paucigranulocytic	0.45	0.00	0.0027	0.00	0.0027	-
	Eosinophilic	0.35	0.42	0.3173	0.68	0.0196	0.1573
	Neutrophilic	0.10	0.05	0.3173	0.05	0.3173	-
	Mixed granulocytic	0.10	0.53	0.0047	0.27	0.1797	0.1573
Ragweed		*n* = 15	*n* = 16		*n* = 14		
	Paucigranulocytic	0.60	0.19	0.0082	0.07	0.0082	0.1573
	Eosinophilic	0.13	0.50	0.0143	0.71	0.0082	0.0833
	Neutrophilic	0.13	0.00	0.1573	0.07	1	0.3173
	Mixed granulocytic	0.13	0.31	0.0833	0.14	-	0.1573
HDMDF		*n* = 10	*n* = 12		*n* = 12		
	Paucigranulocytic	0.10	0.00	0.3173	0.17	0.5637	0.1573
	Eosinophilic	0.60	0.58	1	0.67	0.6547	0.6547
	Neutrophilic	0.30	0.00	0.0833	0.00	0.0833	-
	Mixed granulocytic	0.00	0.42	0.0455	0.17	0.3173	0.0833

Table 3.3. Proportion of sputum inflammatory subtypes before (-24 h) and after (7 h and 24 h) an allergen inhalation challenge.

§, vs. -24 h; †, vs. 7h.

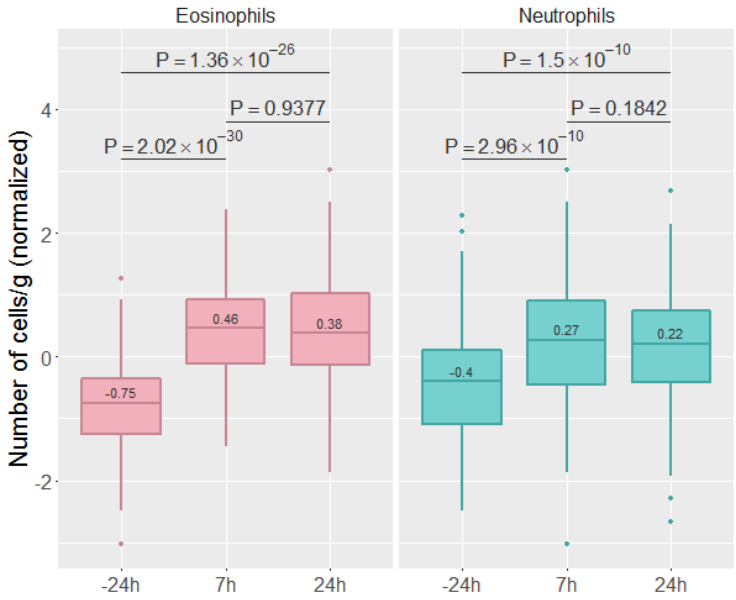


Figure 3.2. Eosinophil and neutrophil cell counts in sputum collected before (-24 h) and after (7 h and 24 h) allergen inhalation challenge.

Sample sizes are summarized in Table 3.3 ('All allergens' group).

At 7 h post challenge, both eosinophils and neutrophils in sputum increased significantly (Figure 3.2; Table 3.4), causing the inflammatory subtype to change for most subjects (62%; Table 3.5 and Supplementary Table 3.1). For example, of the 44 individuals with paucigranulocytic sputum at baseline, 48% and 43% had eosinophilic and mixed granulocytic subtype at 7 h post challenge, respectively. Overall, relative to baseline, there was an increase in the proportion of individuals with the eosinophilic (43% to 55%) and mixed granulocytic (9% to 38%) subtypes, and a decrease in the proportion of individuals with the paucigranulocytic (38% to 6%) and neutrophilic subtypes (9% to 1%) (Figure 3.3, Table 3.3).

		7 h post challenge			24 h post challenge		
	Allergen	N	Δ	*P*-value	N	Δ	*P*-value
Eosinophils	All allergens	112	1.2	$2.0x10^{-30}$	113	1.1	$1.4x10^{-26}$
	Grass	18	1.6	$1.4x10^{-6}$	20	1.3	$6.5x10^{-6}$
	Ragweed	15	1.4	$5.8x10^{-5}$	13	1.2	$5.0x10^{-4}$
	HDMDF	10	1.2	0.0032	10	1.3	0.0012
	HDMDP	35	1.2	$4.5x10^{-11}$	36	1.5	$3.9x10^{-10}$
	Cat	34	1.0	$8.4x10^{-10}$	34	0.8	$1.5x10^{-7}$
Neutrophils	All allergens	112	0.7	$3.0x10^{-10}$	113	0.6	$1.5x10^{-10}$
	Ragweed	15	0.7	0.0923	13	0.3	0.0473
	Grass	18	0.7	0.0056	20	0.6	0.0123
	Cat	34	0.7	$2.7x10^{-5}$	34	0.6	$3.0x10^{-4}$
	HDMDP	35	0.7	$2.0x10^{-4}$	36	0.7	$7.0x10^{-4}$
	HDMDF	10	0.2	0.2385	10	0.5	0.0278

Table 3.4. Change in eosinophil and neutrophil cell counts from baseline (-24 h) to 7 and 24 h post allergen inhalation challenge.

Differences from baseline were calculated based on normalized cells counts per gram of sputum. Abbreviations: Δ, mean difference from baseline.

		Baseline				
		Paucigranulocytic	Eosinophilic	Neutrophilic	Mixed granulocytic	Total at 7h
7 h post challenge	Paucigranulocytic	9%	0%	0%	0%	4
	Eosinophilic	48%	69%	27%	60%	64
	Neutrophilic	0%	0%	9%	0%	1
	Mixed granulocytic	43%	31%	64%	40%	45
	Baseline total	44	49	11	10	114

Table 3.5. Proportion of sputum inflammatory subtypes observed 24 h before allergen inhalation challenge, stratified by the sputum subtype developed 7 h post challenge.

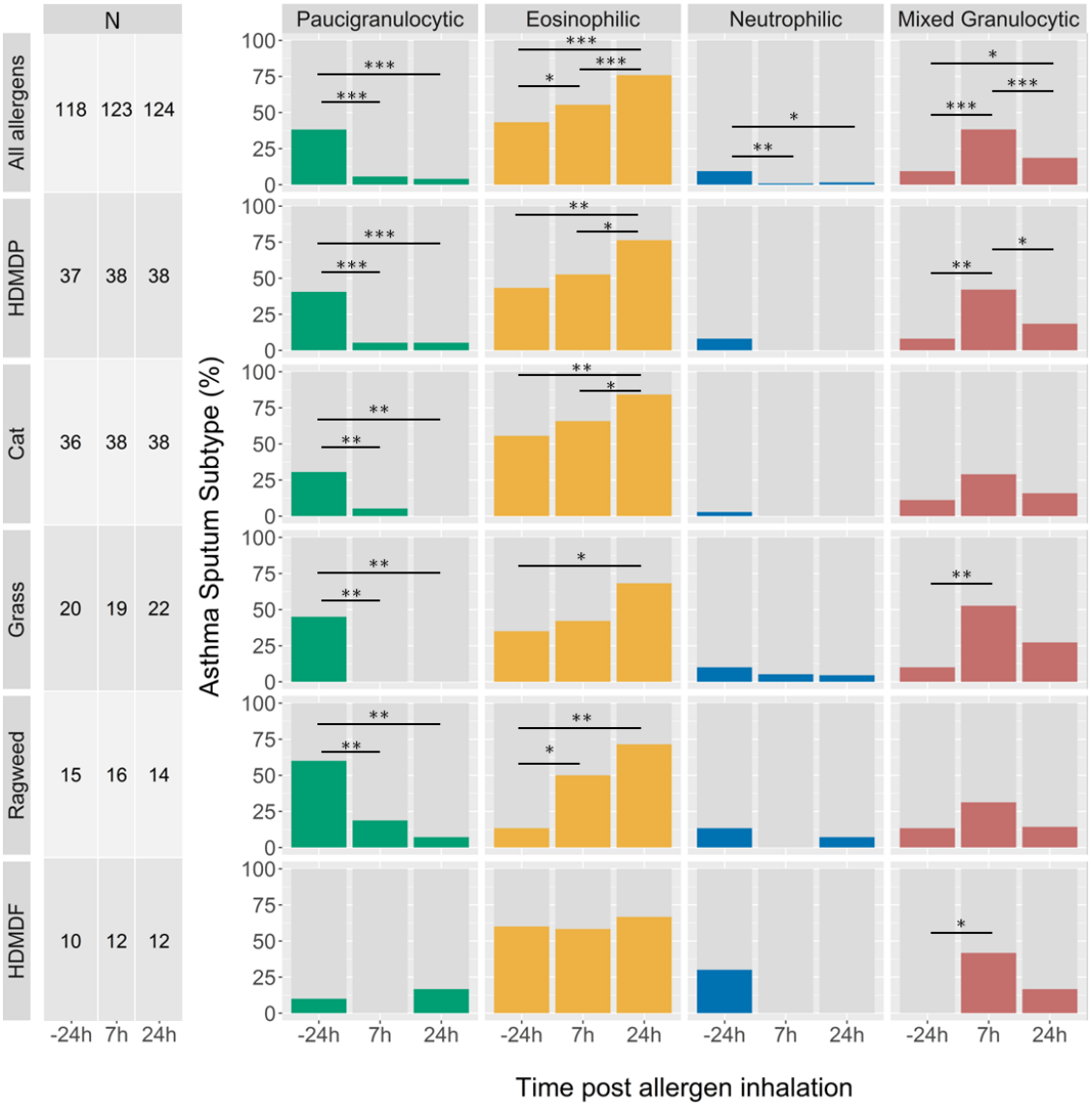


Figure 3.3. Proportion of sputum inflammatory subtypes before (-24 h) and after (7 h and 24 h) an allergen inhalation challenge. Sample sizes for each time point are shown on the left.
** P < 0.05, ** P < 0.01, *** P < 0.001.*

At 24 h post challenge, eosinophils and neutrophils in sputum remained elevated compared to baseline, and largely unchanged from 7 h post challenge (Figure 3.2; Table 3.4). However, when cell counts were assessed as a fraction of the total cell count, there was a significant decrease in the proportion of neutrophils from 7 h to 24 h post challenge (Figure 3.4). This resulted in a significant decrease in the proportion of individuals with the mixed granulocytic subtype (38% to 19%) at 24 h, and a significant increase in the proportion of individuals with the eosinophilic subtype (55% to 76%) (Figure 3.3 and Table 3.3).

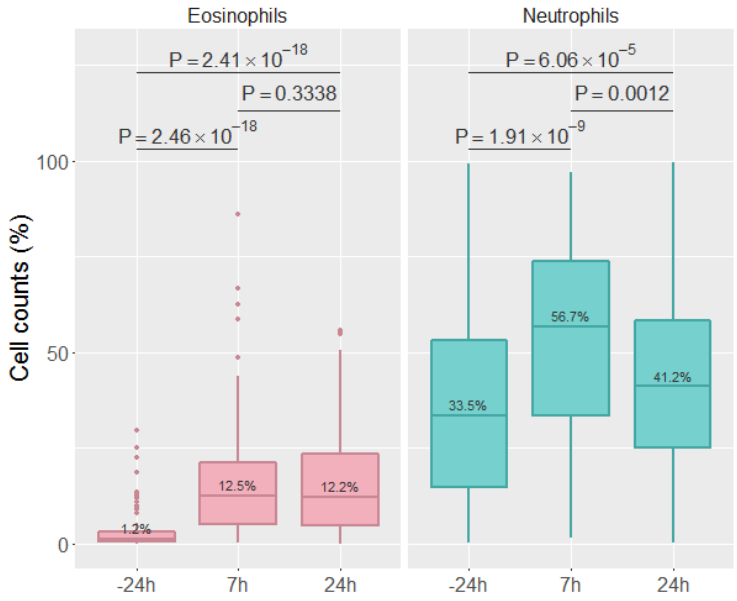


Figure 3.4. Eosinophils and neutrophil cell proportions in sputum collected before (-24h) and after (7 h and 24 h) allergen inhalation challenge.

Sample sizes are summarized in Table 3.3 ('All allergens' group).

When considering the difference in sputum cell counts between the baseline and post-challenge time points, which reflects how many cells entered the airways after the challenge, there was no association between the severity of the LAR and the number of eosinophils or neutrophils (Table 3.6). However, individuals with higher eosinophil (P = 0.014) and neutrophil (P = 0.018) sputum counts at baseline developed a more severe LAR upon allergen challenge (Table 3.6).

	N	β	*P*-value
Sputum subtype at baseline	118	NA	0.32388
Neutrophils at baseline	117	0.19	0.01824
Eosinophils at baseline	117	0.26	0.01371
Sputum subtype at 7 h post challenge	123	NA	0.51214
Neutrophils at 7 h post challenge	121	0.03	0.69377
Eosinophils at 7 h post challenge	121	0.16	0.09762
Difference in neutrophils between 7 h and baseline	112	-0.07	0.2989
Difference in eosinophils between 7 h and baseline	112	0.03	0.76726

Table 3.6. Association between the severity of the LAR and sputum granulocyte counts and inflammatory subtype.

Sputum subtype and cell counts were assessed before (-24 h; baseline) and after (7 h) allergen challenge.

Effect of allergen type on sputum granulocyte counts and inflammatory subtype

Compared to baseline, all allergens promoted a significant (Figure 3.5) and comparable (Figure 3.6 and Table 3.4) increase in sputum eosinophil counts, both at 7 h and 24 h post challenge.

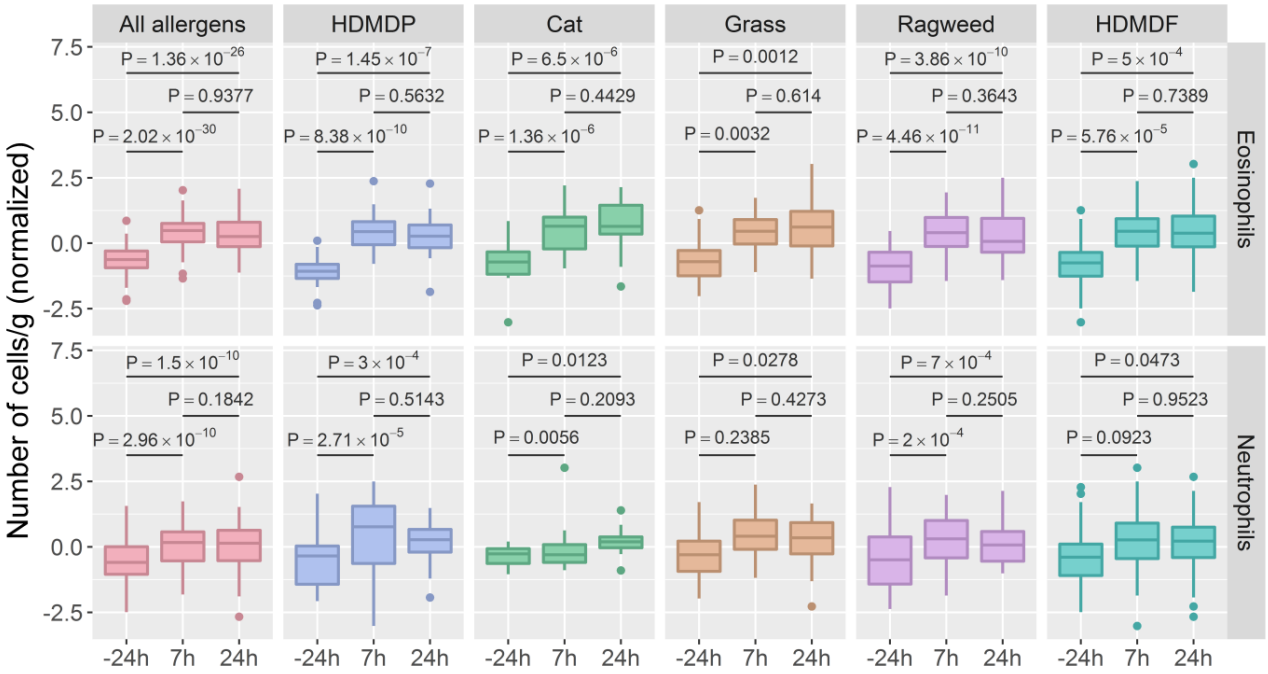


Figure 3.5. Eosinophil and neutrophil cell counts in sputum collected before (-24 h) and after (7 and 24 h) allergen inhalation challenge, stratified by allergen.

Sample sizes are summarized in Table 3.3.

On the other hand, neutrophil counts only increased significantly at 7 h post challenge for HDMDP, cat and grass allergens (Figure 3.5), although a similar trend was observed with HDMDF and ragweed (Figure 3.6 and Table 3.4). At 24 h post challenge, all allergens promoted a significant increase in neutrophils, suggesting a delayed infiltration of neutrophils with some allergens.

At 7 h post challenge, the eosinophilic subtype was found at a similar frequency across allergens, ranging from 42% of individuals tested with grass to 66% of those tested with cat (Figure 3.3 and Table 3.3). The second most common subtype at this time point, mixed granulocytic sputum, also occurred with similar frequency across allergen types, ranging from 29% for cat to 53% for grass allergen. Overall, there were no significant differences in the frequency of sputum inflammatory subtypes between the five allergen types tested (P = 0.298).

At 24 h post challenge, the eosinophilic subtype was found in 66% (HDMDF) to 84% (cat) of individuals, and the mixed granulocytic in 14% (ragweed) to 27% (grass) of individuals, again with no overall statistically significant differences in subtype frequencies between allergen types (P = 0.255).

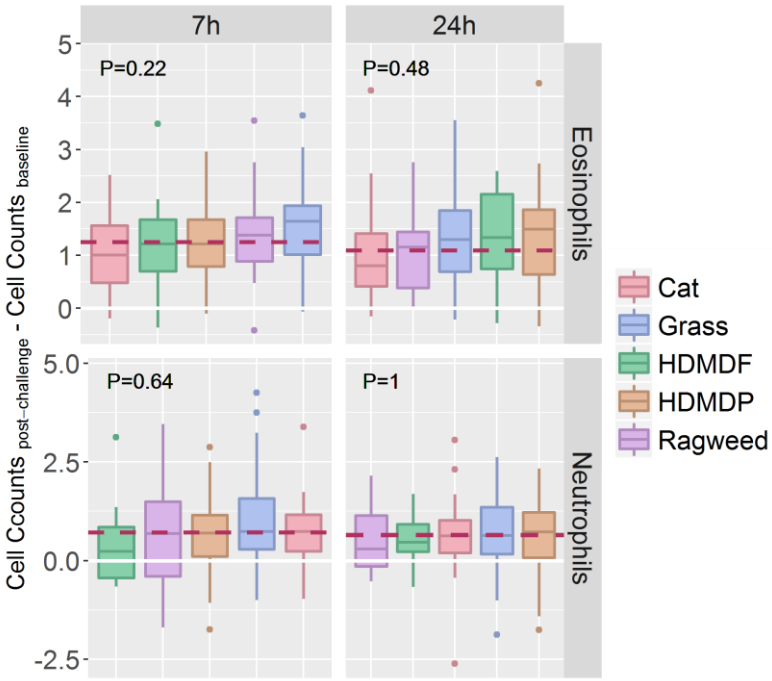


Figure 3.6. Change in sputum eosinophil and neutrophil cell counts from baseline (-24 h) to 7 and 24 h post allergen inhalation challenge, stratified by allergen.

Differences from baseline were calculated based on normalized cells counts per gram of sputum. The dashed line represents the overall median change from baseline. Sample sizes are summarised in Table 3.4.

Discussion

In this study, we analysed data from allergen inhalation challenges to test the hypothesis that different allergen types can induce distinct inflammatory profiles in the airways. The study population included exclusively individuals with mild allergic asthma.

Unexpectedly, we found no association between the number of eosinophils and neutrophils that infiltrate into the airways after allergen challenge and the severity of the LAR. On the other hand, individuals with increased eosinophil and neutrophil sputum counts at baseline had a more severe LAR, consistent with results from previous studies.[159,163] These results suggest that the presence of

larger numbers of granulocytes at baseline, perhaps reflecting ongoing or chronic inflammatory processes, is a risk factor for a more severe asthma exacerbation upon acute allergen exposure. This is not surprising, given the active contribution of eosinophils and neutrophils to asthma immune responses. These cells produce and release proteases, cytokines and chemokines aimed at eliminating pathogens.[164,165] Recurrent release of these mediators contributes to irreversible airway remodelling, which in turn is associated with progressive loss of lung function. More importantly, degranulation of eosinophils and neutrophils releases mediators that recruit immune cells involved in adaptive immune responses, and these have been shown to associate with the magnitude of the LAR.[166] Specifically, the severity of the LAR associates with allergen-specific proliferative response of peripheral T lymphocytes, which in turn are correlated with increased serum levels of IL-5, a cytokine produced by eosinophils.[166]

When we compared the frequency of sputum inflammatory subtypes between individuals challenged with different allergen types, we found no overall significant differences. This is in contrast with findings from studies of experimental acute allergic asthma conducted in isogenic mice. Such differences could arise, for example, if there is a significant genetic contribution to variation in sputum subtype in humans. Blood cell counts are highly heritable in humans, with about 70% of the variation in eosinophil and neutrophil numbers explained by genetic factors.[167] There is also a significant positive correlation in granulocyte counts between blood and sputum, most strongly for eosinophils.[138,168,169] Therefore, it is possible (if not likely) that sputum cell counts are also heritable. On the other hand, environmental factors might also contribute to variation in sputum subtype after acute allergen challenge in humans but not (or less so) in mouse studies. For example, recent studies have shown that depending on the microbiota composition of the lungs, the airway inflammatory phenotype may adopt a neutrophilic- or eosinophilic-like profile.[170-173] The intestinal microbiome can also modulate the allergic responses in the airways through the production of byproducts that regulate cell function and differentiation.[174] Thus, unlike described in mice, allergen type is likely to have no (or only a small) contribution to variation in sputum subtype following acute allergen challenge.

The changes in sputum inflammatory cells seen in the paucigranulocytic subtype warrant some discussion, especially as the pathogenesis of this asthma subtype is not well understood. Our findings indicate that individuals with paucigranulocytic asthma can readily develop an eosinophilic or mixed granulocytic sputum subtype after allergen exposure. This suggests that it is not correct to regard paucigranulocytic asthma as a static phenotype but one in which airway inflammation can develop dynamically in response to sensitizing allergens.

There are a number of limitations that need to be considered when interpreting results from our study. First, the study design used does not explicitly control for many factors that could potentially contribute to inter-individual variation in sputum subtype. This could decrease power to detect a true effect of allergen type on variation in sputum subtype frequency. For example, in mouse studies, animals are isogenic, kept in a comparable environment, fed the same diet and challenged within days of each other. As such, the effect of genetic and (many) environmental factors on sputum subtype variability is minimized, which was not the case in our study. Therefore, in mice, allergen type might explain a larger fraction of sputum subtype variability, and so such effect might be detectable with relatively small sample sizes. A comparable and more desirable study design in humans would be to test if different allergens trigger different sputum subtypes in the same individual, with different allergens tested in a relatively narrow time frame. Such data were available for only a small number of individuals in our study, and so were not considered in our analyses. Second, to facilitate the analysis and interpretation of results, we averaged observations from individuals who completed multiple allergen challenges using the same allergen. Although this is unlikely to have affected the robustness of our findings, a more powerful approach would have been to use mixed models and include random effects to account for the presence of non-independent observations. We explored this approach but often had convergence problems, thus questioning the validity of the model estimates. Nonetheless, with this caveat in mind, results obtained with mixed models were consistent with our conclusions (not shown). Third, not all allergens tested in these analyses were equally represented. HDMDP, the most common allergen in the dataset, was used in 40 allergen inhalation tests, whereas HDMDF, the rarest allergen in the dataset, was only used in 12 allergen inhalation tests. Small sample sizes could affect the power to detect differences between allergens. Another caveat relates to the allergen groupings used in our analysis. We opted to analyse the two HDM species separately (instead of combining them into a broader HDM group) because known differences between these allergens [175,176] could potentially trigger distinct immunological responses. The same holds for the two types of pollen that were analysed separately in our study (ragweed and grass). When we repeated our analyses with only three major allergen groups (HDM, pollen and cat), results remained largely unchanged (not shown). Lastly, the outcome of interest in these analyses was the sputum subtype, which is defined by the proportion of eosinophils and neutrophils in sputum. The thresholds we used to classify sputum subtype were proposed by Simpson et al,[124] corresponding to the 95th percentiles observed in healthy individuals. However, other studies have proposed slightly different thresholds,[138,177,178] which might result in a different sputum subtype classification for some individuals in our study. Which thresholds are more appropriate to define the different sputum

subtypes is still an open question. However, when we categorized sputum subtype according to other published thresholds, results remained unchanged, with no significant effect of allergen type on the frequency of sputum subtypes (not shown).

In conclusion, we found no association between the type of allergen used in inhalation challenge tests and the sputum inflammatory subtype developed. This observation is important because it suggests that there are no specific allergen types that can be used reliably to enrich allergen-induced responses for an inflammatory subtype of interest, as might be important in clinical trials of biologic therapies for asthma.

Chapter 4

Genes co-expressed with *IL6R* that also associate with asthma risk

CHAPTER 4. GENES CO-EXPRESSED WITH *IL6R* THAT ALSO ASSOCIATE WITH ASTHMA RISK

The goal of this chapter was to increase our understanding of gene networks that are perturbed in asthma by identifying genes that are (1) co-expressed with *IL6R* and (2) associated with asthma risk. Results from these analyses were published in the Allergy journal in 2016:

Revez JA, Matheson MC, Hui J, Baltic S, Australian Asthma Genetics Consortium c, James A, Upham JW, Dharmage S, Thompson PJ, Martin NG, Hopper JL, Ferreira MA: Identification of *STOML2* as a putative novel asthma risk gene associated with IL6R. Allergy 2016;71:1020-1030.

Introduction

Chapter 1 reviewed extensive evidence from both human genetic association studies[1,26,122] and experimental animal models of asthma[32,33] that suggested that dysregulation of *IL6R* expression contributes to asthma pathophysiology. However, it is important to recognize that *IL6R* expression is also associated the expression of other immune and inflammatory genes.[179,180] For instance, in a study that evaluated the contribution of IL-6 to obesity-related inflammation, gene expression levels measured in adipose tissue showed that *IL6R* expression was positively correlated with the expression of inflammatory genes involved in processes such as insulin resistance, chemotaxis and infiltration of inflammatory cells into adipose tissue.[180]

Thus, in asthma, the expression of *IL6R* may also be associated with the expression of other genes that simultaneously contribute to increased disease risk. To our knowledge, this possibility has not been studied systematically to date. To address that hypothesis, we first analysed two publicly available gene expression datasets to search for genes whose expression was correlated with that of *IL6R* (henceforth referred to as "*IL6R*-associated genes"). Then, this information was integrated with results from both asthma and transcriptome GWAS to identify the subset of *IL6R*-associated genes that are likely to be genetic risk factors for asthma. Identifying disease risk genes that are also associated with *IL6R* might provide new clues into the broader gene network that underlies the pathological effect of IL-6 signalling dysregulation in asthma.

Methods

Our analytical procedure is summarized in Figure 4.1 and described in detail below.

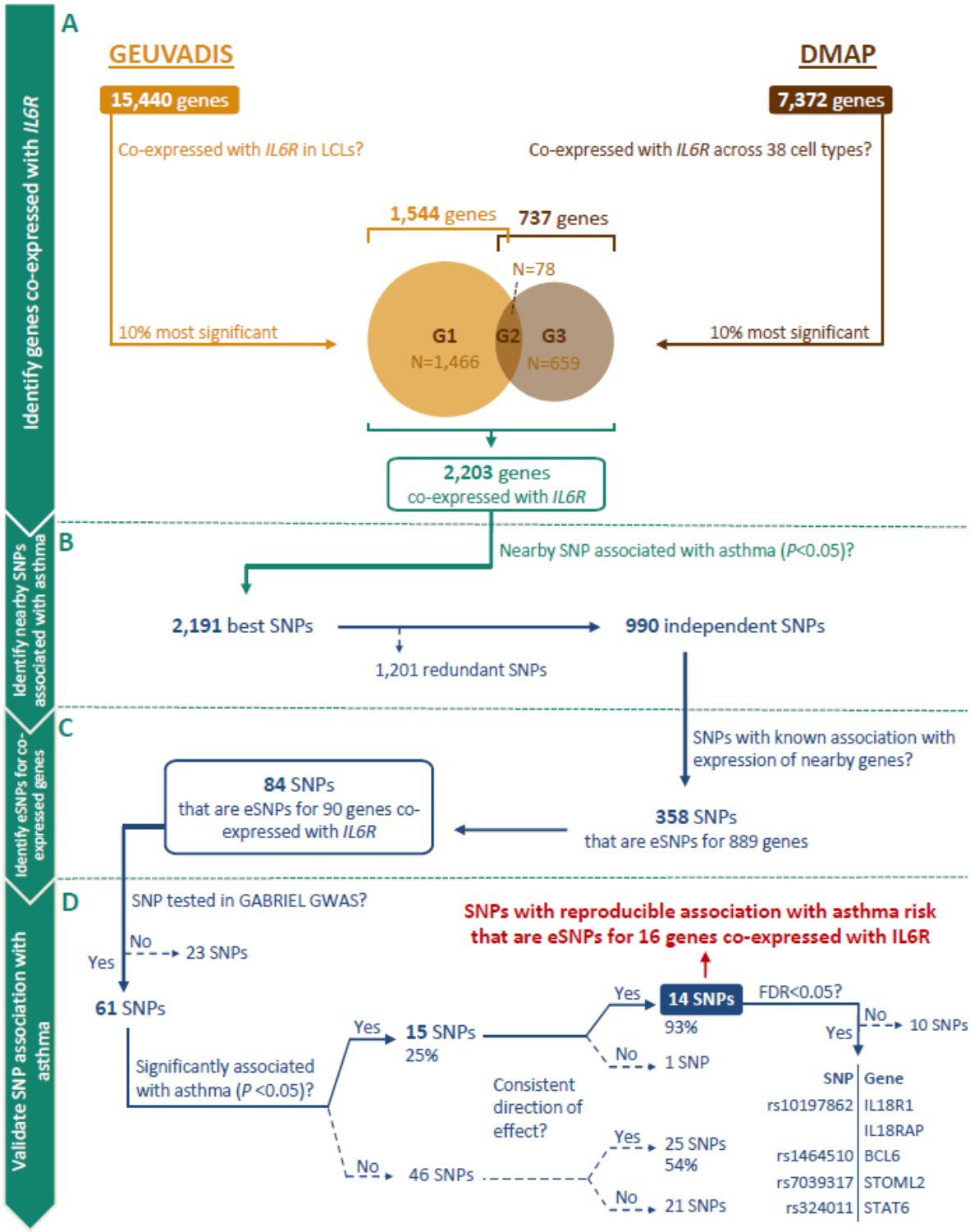


Figure 4.1. Flowchart of IL6R co-expression analysis.

Steps taken for the identification of genes with expression (i) associated with that of IL6R and (ii) regulated by asthma risk SNPs.

Analysis of gene expression levels in 373 Europeans studied by the Geuvadis consortium

To identify genes with expression levels correlated with those of *IL6R*, we first analysed RNA-seq data generated by the Geuvadis consortium for human lymphoblastoid cell lines (LCLs)[181]. LCLs are derived from peripheral blood B-cells and so represent a practical *in vitro* model to study gene expression patterns relevant to immune-related conditions.

We downloaded from the European Bioinformatics Institute (EBI) RPKM-normalized gene expression levels for 53,934 transcripts measured in LCLs of 462 unrelated individuals from the 1000 Genomes Project (accession E-GEUV-1, file GD660.GeneQuantRPKM.txt.gz). Transcripts expressed in > 90% of individuals (N = 18,702) were selected and distributions normalized using a rank-based inverse-normal transformation. We restricted our analysis to samples of European ancestry (N = 373) and unique transcripts (unambiguous match between HGNC symbol and corresponding Ensembl ID) annotated in GENCODE[182] (N = 15,440). The association between *IL6R* expression levels and the expression of each of the other 15,440 genes was then tested using linear regression, with covariates included in the model to adjust for the effects of sex, population (CEU, GBR, FIN, TSI), sequencing centre and gene GC content (see below). Sixteen principal components (PCs) were also included as covariates to account for the effect of unmeasured confounders, as described below. These analyses were performed in R version 3.2.2.

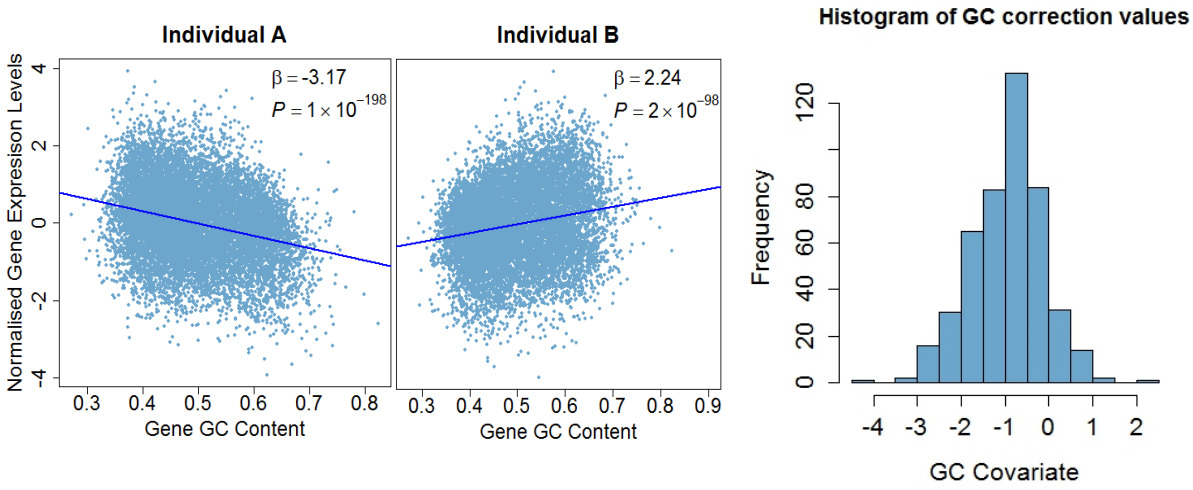


Figure 4.2. Comparison of GC content effect on gene expression levels of two representative individuals (two left panels) and histogram of GC covariate values.

Histogram of GC correction values is based on sample of 462 individuals.

Gene GC content can affect RNA-seq-derived expression levels differently in different individuals.[183,184] We tested this possibility and indeed found that in some individuals, increased gene GC content was associated with increased gene expression, while in others the reverse was true (Figure 4.2). To correct for this potential confounder, we calculated a GC correction per individual as follows. From the 18,702 genes expressed in more than 90% of individuals, we (1) excluded a subset of 3,756 highly expressed genes (RPKM > 21.48); (2) normalized the distribution of the remaining genes with a rank-based inverse-normal transformation; and (3) for each individual determined a correction value as the regression slope obtained from the regression of gene expression levels on gene GC content (for two examples, see Figure 4.2). GC content for each gene was obtained from the Conditional Quantile Normalization R package for 15,277 genes.[183] To correct for the effects of unmeasured confounders, PC analysis was applied to the matrix of quantile-normalized gene expression levels using the prcomp function of the R stats package,[185] and the first 16 PCs were then extracted and included as covariates when testing the association between *IL6R* expression levels and the expression levels of other genes.

The top 10% of genes with expression most associated with that of *IL6R* were then selected for subsequent analyses. For a justification of how/why we selected a cut-off of 10% (instead of e.g. 5%), see section "Selection of cut-off to prioritize *IL6R*-associated genes" below.

Analysis of gene expression levels in 38 cell types from the DMAP project

We used a second independent approach to identify genes associated with *IL6R*. This approach differed from the analysis of the Geuvadis data in that we searched for genes correlated with *IL6R* within an individual but across immune cell types (e.g. cell types with increased expression of *IL6R* also have increased expression of gene X), instead of within a cell type but across individuals (e.g. individuals with increased expression of *IL6R* in LCLs also have increased expression of gene X).

A file (DMap_data.gct) containing normalized gene expression levels for 8,968 genes measured in 38 hematopoietic cell populations isolated from four to seven individuals was downloaded from the Differentiation Map Portal (DMAP) project[186] website. We then selected 7,372 unique genes annotated in GENCODE,[182] and measured the association of their expression with *IL6R* expression using the cosine distance metric, as implemented in the GeneNeighbors module of GenePattern.[187] As for the Geuvadis data, the top 10% of genes most associated with *IL6R* expression (based on the absolute of the distance metric) were selected for subsequent analyses.

Identification of SNPs associated with asthma risk and variation in gene expression

Not all genes associated with *IL6R* will contribute to asthma pathophysiology. To identify the subset of *IL6R*-associated genes whose expression might be causally-related to asthma risk, we combined information from published GWAS of asthma and published GWAS of gene expression levels. Specifically, for each gene X (e.g. *BCL6*) that is associated with *IL6R*, we (1) extracted summary association statistics (effect, risk allele, *P*-value) for SNPs within 1 Mb of the gene boundaries from the Ferreira et al. GWAS,[188] which included 6,685 asthmatics and 14,091 controls; (2) selected the SNP with the most significant association with asthma (amongst those with $P < 0.05$) in that 1 Mb interval; (3) identified all genes whose expression was previously reported to associate (typically, but not always, with FDR < 0.05 imposed by the original study) with this SNP (or a proxy, $r^2 > 0.8$) in 11 published GWAS of gene expression levels conducted in cells/tissues relevant to asthma[181,189-198]; and (4) retained that SNP for further analysis if one of the genes known to be regulated by this SNP was also gene X (i.e. *BCL6*). In other words, we selected a subset of *IL6R*-associated genes that are regulated by a nearby SNP that associates with asthma risk.

We selected this approach for two main reasons. First, disease risk variants that associate with gene expression are more likely to reflect causal associations. This has been demonstrated in several studies,(23650636) including a study by Moffat et al. that showed that genome-wide significant variants associated with childhood asthma were consistently associated with the expression of *ORMDL3*,[59] a gene that was subsequently shown to be inducible in lung epithelial cells and involved in airway remodelling.[199,200] Second, we restricted our analysis to cis eQTLs because most GWAS of gene expression used here (performed in tissues relevant for asthma, e.g. B cells) had low power to detect trans effects, or reported cis effects only.

We considered an alternative more comprehensive analytical strategy (selection of all SNPs associated with asthma in step (2) above), but found that this would result in hundreds of SNPs being carried forward to validation, which would decrease power given the multiple testing burden (see below).

Selection of cut-off to prioritize IL6R-associated genes for downstream analyses

In the first step of our analytical procedure (Figure 4.1 A), we used a 10% cut-off to select a group of genes whose expression was most strongly associated with that of *IL6R*. The choice of cut-off determined the number of genes selected for downstream analysis, and so also the number of SNPs that were moved to validation (see below). As such, an important consideration when deciding what

cut-off to use was the power provided by the validation study to replicate a significant association after accounting for multiple SNP testing. Cut-offs of 5% and 10% resulted in 37 and 61 SNPs reaching the validation step, respectively. Based on the specific effect sizes and minor allele frequencies observed for these SNPs in the discovery GWAS, we estimated the power provided by the validation study to replicate the associations at a Bonferroni-corrected threshold of 0.0014 (0.05/37) and 0.0008 (0.05/61), respectively. The validation study was adequately powered to replicate 22 of the 37 (59%) SNP associations obtained with the 5% cut-off and 35 of the 61 (57%) associations obtained with the 10% cut-off. Therefore, the ability to replicate the associations with these two sets of SNPs was comparable between the two cut-offs (59% vs. 57%). The 10% cut-off maximised the opportunity to discover new risk SNPs, and so we selected that cut-off.

Validation of genetic associations with asthma risk

The previous analyses identified a set of SNPs found to regulate an *IL6R*-associated gene and that were also associated with asthma risk in the Ferreira et al. GWAS.[188] We refer to these SNPs that are associated with variation in the expression of a nearby gene as "eSNPs".

Some of these might represent novel risk variants for asthma. To test this possibility, we investigated whether these eSNPs were also associated with asthma risk in an independent study, the GABRIEL consortium GWAS.[62] After excluding overlapping samples (N = 1,207, Busselton cohort), results were based on the analysis of 12,077 asthmatics and 15,301 controls. A reproducible association was defined as a significant ($P < 0.05$) and consistent (i.e. same direction of effect as in the Ferreira et al. GWAS[188] association in this analysis. The proportion of eSNPs for *IL6R*-associated genes with a reproducible association with asthma out of all eSNPs tested in the GABRIEL is denoted in the section below by $f_{IL6R\text{-}genes}$. To obtain an overall measure of association between an eSNP and asthma risk, we used METAL[201] to meta-analyse results from the Ferreira et. al GWAS and the GABRIEL GWAS using a fixed-effects model.

Comparison with a random selection of genes

We applied the same analytical approach described above (Figure 4.1) to a random set of 1,000 genes, instead of *IL6R*. This allowed us to estimate the extent to which the enrichment of significant associations between asthma risk and eSNPs for *IL6R*-associated genes was because eSNPs in general – and not just those specific to *IL6R*-associated genes – are more likely to be disease-associated than randomly selected SNPs.[202] To this end, we (1) selected a random gene tested in both the Geuvadis and DMAP datasets; (2) applied steps A through D (Figure 4.1) to identify genes co-expressed with

that random gene (instead of *IL6R*); and (3) identified the proportion of eSNPs for genes co-expressed with that random gene that had a reproducible association asthma risk (and denote this fraction by $f_{random\text{-}genes}$). This procedure was repeated for 1,000 different random genes. We then calculated the mean $f_{random\text{-}genes}$ across the 1,000 replicates, and the proportion of replicates where $f_{random\text{-}genes} \geq f_{IL6R\text{-}genes}$. This proportion represents the (empirically-derived) probability of observing reproducible eSNP associations with asthma risk more often than observed for *IL6R*-associated genes when selecting a random gene of interest.

Results

Identification of genes with expression levels associated with those of IL6R

To identify genes that were associated with *IL6R*, we used two complementary approaches (Figure 4.1 A). First, we tested the association between *IL6R* expression and that of 15,440 genes measured with RNA-seq in LCLs of 373 individuals of European descent by the Geuvadis consortium.[181] In this analysis, the expression of 1,020 genes (6.6%) was associated with the expression of *IL6R* at a $P < 0.05$. The most associated genes (N = 1,544; top 10%) are listed in Supplementary Table 4.1 and include notable genes such as *ATP8B2* and *IL18R1*, which are respectively a gene located near *IL6R* and a gene located in an established asthma risk locus.[203]

The previous analysis aimed to identify genes associated with *IL6R* when comparing expression levels measured in a single cell type across different individuals. In a separate approach, we analysed data from the DMAP project[186] to search for genes whose expression values were correlated with *IL6R* across 38 different hematopoietic cell populations. In this analysis, the expression of 3,643 genes (49%) was associated with that of *IL6R* at $P < 0.05$. This excess of significant associations likely arises because cell types analysed by the DMAP project derive from a common hematopoietic progenitor cell and many belong to the same lineage, and so their transcriptional profiles are highly correlated.[186] As for the analysis of the Geuvadis data, we then selected the top 10% of genes with expression patterns most associated with *IL6R* for subsequent analysis (N = 737, Supplementary Table 4.1). Amongst the most *IL6R*-associated genes were for example *LY96* and *TLR2*, two genes involved in the regulation of inflammation.[204,205]

After combining the lists of genes most associated with *IL6R* in these two independent approaches (top 10% of each), we obtained 2,203 genes (Figure 4.1 A), including those identified (1) in the Geuvadis dataset only (N = 1,466); (2) in both the Geuvadis and DMAP datasets (N = 78) and (3) in the DMAP dataset only (N = 659).

We used the STRING v10[206] online database to analyse the 78 genes identified through both approaches, and found a significant enrichment for cellular components involved in binding and present in the cell periphery or plasma membrane. In addition, this set of genes was enriched in biological processes involved in cellular response to stimulus, suggesting that, like *IL6R*, the genes identified through the GEUVADIS and DMAP studies code for proteins involved in cell signalling.

Identification of asthma risk SNPs near IL6R-associated genes

The analysis above identified 2,203 genes with expression levels associated with *IL6R*, either across different individuals and/or cell types. We hypothesized that the expression of some of these genes might be causally related to asthma. If so, we would expect that genetic polymorphisms that regulate the expression of these genes would be associated with asthma risk. To test this possibility, for each gene, we first identified the nearby (+/- 1 Mb) single nucleotide polymorphism (SNP) with significant ($P < 0.05$) and strongest association with asthma risk in a recently published asthma GWAS,[188] that included 6,685 cases and 14,091 controls. For twelve genes, there were no significant SNPs within 1 Mb, and so these were not considered further. The resulting list of 2,191 SNPs was further pruned by removing duplicate (i.e. same SNP selected for two or more genes; N = 1,122) or correlated (linkage disequilibrium $r^2 > 0.1$; N = 79) SNPs, leaving for further analysis 990 independent SNPs that were associated with asthma risk and located near an *IL6R*-associated gene.

Regulation of gene expression by asthma risk SNPs near IL6R-associated genes

We then investigated if the association between each of these 990 independent SNPs and asthma risk could arise because these SNPs regulate the expression of the nearby *IL6R*-associated gene. Of the 990 SNPs, 358 (36%) were associated with the expression of at least one nearby gene in published GWAS of gene expression levels (which we refer to as eSNPs), including 84 (8.5%) that were eSNPs for the actual gene that was correlated with *IL6R* (Supplementary Table 4.2).

In total, the 84 eSNPs associated with the expression of 90 *IL6R* co-expressed genes. When we analysed these genes with the STRING v10[206] online database, we observed a significant enrichment for genes involved in lymphocyte and leukocyte activation. In addition, these genes were enriched for adaptive immune responses and regulation of isotype switching to IgE isotypes.

Replication of the association between asthma and eSNPs for IL6R-associated genes

Eighty-four eSNPs were associated with both asthma risk in the Ferreira et al. GWAS[188] and expression levels of an *IL6R*-associated gene in published GWAS of gene expression. To validate the

association observed between asthma risk and these 84 eSNPs, we analysed publically available results from the GABRIEL asthma GWAS.[62] After excluding samples that overlapped with the Ferreira et al. GWAS, results were available for 12,077 asthmatics and 15,301 controls. Of the 84 eSNPs, 61 were tested in the GABRIEL GWAS and for 14 of these (23%) there was a significant ($P < 0.05$) and consistent (same direction of effect) association (Table 4.1 and Figure 4.1), when only about two reproducible associations were expected at this significance level by chance alone given multiple testing (61 [eSNPs tested] x 0.05 [$P < 0.05$] x 0.5 [same direction] = 1.5).

			Co-expression with *IL6R*			Ferreira, 2014		GABRIEL		
No.	eSNP	Chr:bp	Gene	Cor	Study	OR, allele	*P*-value	Proxy SNP	OR*, allele	P-value
1	**rs10197862**	**2:102350089**	***IL18R1***	**-0.202**	**G**	**1.24, A**	**$4x10^{-11}$**	**rs13431828**	**1.2, C**	**$2x10^{-9}$**
	rs10197862	**2:102350089**	***IL18RAP***	**-0.143**	**G**	**1.24, A**	**$4x10^{-11}$**	**rs13431828**	**1.2, C**	**$2x10^{-9}$**
2	**rs1464510**	**3:188394766**	***BCL6***	**0.324**	**D**	**0.91, A**	**0.0001**	**rs1559810**	**0.92, A**	**0.0001**
3	**rs7039317**	**9:34964538**	***STOML2***	**-0.346**	**D**	**1.11, T**	**0.0003**	**-**	**1.09, T**	**0.0010**
4	**rs324011**	**12:57108399**	***STAT6***	**0.415**	**D**	**1.09, T**	**0.0001**	**rs167769**	**1.07, T**	**0.0022**
5	rs4833095	4:38798089	*TLR1*	0.448	D	1.2, T	$5x10^{-12}$	-	1.07, T	0.0044
6	rs2357792	16:50734311	*NOD2*	0.648	D	1.08, A	0.0011	rs6500331	1.06, G	0.0050
7	rs13416555	2:8301605	*ID2*	0.324	D	1.12, C	$1x10^{-5}$	rs10178845	1.06, G	0.0120
8	rs6511788	19:12369223	*MAN2B1*	0.425	D	1.07, T	0.0088	-	1.06, T	0.0160
9	rs11265424	1:160530060	*SLAMF1*	0.109	G	0.91, A	0.0005	rs1055880	0.95, C	0.0178
10	rs2435206	17:45980745	*NSF*	0.374	D	1.08, T	0.0014	rs2435211	1.05, T	0.0197
11	rs1253118	14:59497301	*RTN1*	0.556	D	1.07, T	0.0051	rs9323348	1.05, T	0.0210
12	rs13212921	6:27237643	*BTN2A1*	0.329	D	1.14, T	0.0002	rs13219354	1.08, C	0.0224
13	rs11000805	10:73903593	*NDST2*	0.349	D	1.07, C	0.0145	rs17741873	1.06, T	0.0316
14	rs9289837	3:151388204	*P2RY13*	0.452	D	0.88, T	$1x10^{-5}$	rs7637803	0.95, T	0.0416
	rs9289837	3:151388204	*MED12L*	0.129	G	0.88, T	$1x10^{-5}$	rs7637803	0.95, T	0.0416

Table 4.1. Fourteen SNPs that are eSNPs for genes that are co-expressed with IL6R and also have a reproducible association with asthma risk.

*Highlighted in light grey are SNPs that had not previously been reported to associate with asthma risk. SNPs with significant association with asthma after correction for multiple testing (FDR < 0.05) in the replication analysis are represented with bold font. *The OR is reported for the allele that is on the same haplotype as (i.e. in phase with) the allele reported for the Ferreira, 2014 GWAS SNP. Proxy SNPs were chosen based on an $r^2 > 0.8$. Abbreviations: BP, base pair; Chr, chromosome; D, DMAP; G, Geuvadis; OR, Odds Ratio*

SNP	A1	A2	Effect	SE	*P*-value	Direction
rs10197862	A	G	0.197	0.0222	$7.25x10^{-19}$	++
rs4833095	T	G	0.121	0.0179	$1.67x10^{-11}$	++
rs1464510	A	G	-0.086	0.0153	$1.70x10^{-08}$	--
rs324011	T	G	0.077	0.0154	$5.80x10^{-07}$	++
rs13416555	C	G	0.082	0.017	$1.54x10^{-06}$	++
rs7039317	T	G	0.094	0.0196	$1.67x10^{-06}$	++
rs9289837	T	G	-0.085	0.0194	$1.20x10^{-05}$	--
rs2357792	A	G	0.068	0.0158	$1.82x10^{-05}$	++
rs13212921	T	G	0.102	0.024	$2.00x10^{-05}$	++
rs11265424	A	G	-0.068	0.0169	$5.63x10^{-05}$	--
rs2435206	T	G	0.062	0.0162	0.00015	++
rs1253118	T	G	0.056	0.0157	0.00033	++
rs6511788	T	G	0.063	0.0175	0.00034	++
rs11000805	C	G	0.064	0.0192	0.00094	++

Table 4.2. Meta-analysis of the Ferreira 2,014 and GABRIEL GWAS for the 14 SNPs with validated asthma risk association.

Abbreviations: A1, allele 1; A2, allele 2; SE, standard error.

Of the 14 eSNPs (representing 16 genes; Figure 4.3) with a reproducible association with asthma risk in the replication analysis, four were significant after controlling for the 61 SNPs tested (FDR[207] < 0.05). Three of these are established risk variants for allergic disease, specifically those regulating the expression of *IL18R1*/*IL18RAP*,[62] *BCL6* and *STAT6*.[208] On the other hand, rs7039317 (combined discovery and replication analysis OR = 1.10 for the T allele, $P = 2 \times 10^{-6}$; Table 4.2), an eSNP for *STOML2*, has not previously been reported to be associated with the risk of asthma or other allergic diseases. Therefore, *STOML2* represents a putative novel risk gene for asthma.

To get mechanistic insight into the function of the *STOML2* eSNP (rs7039317), we queried HaploReg v4,[209] an online tool that combines information from several GWAS annotation resources, including the Roadmap Epigenomics and ENCODE projects. We found that the *STOML2* eSNP overlaps an enhancer region in skin epithelial cells. Thus, it is possible that rs7039317 affects the binding of a transcription factor that then determines the transcription levels of *STOML2*, as has been postulated for other non-coding variants identified through GWAS.

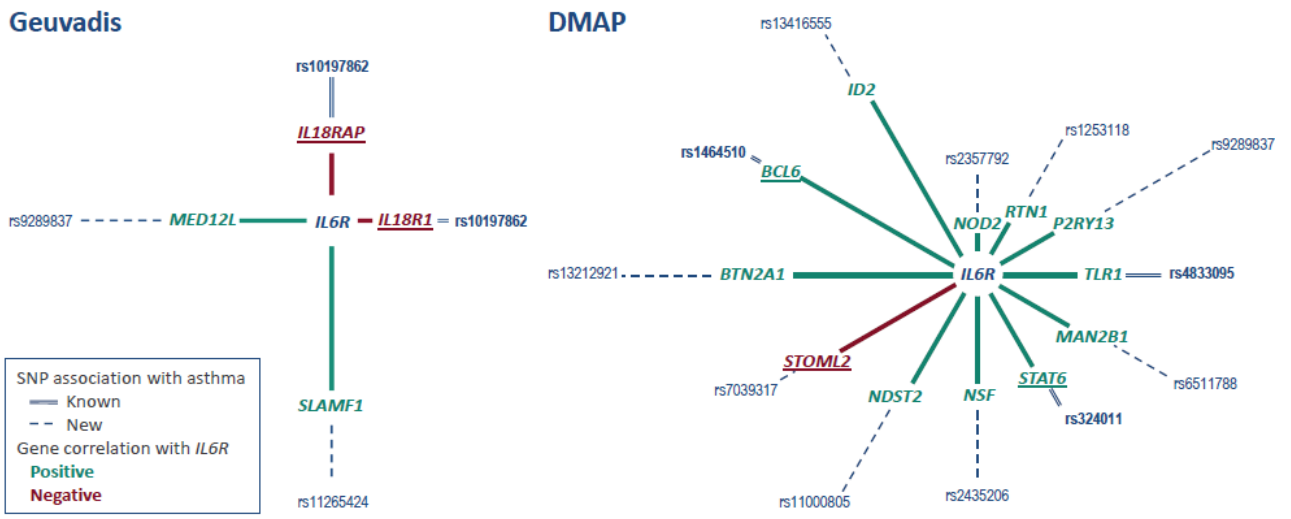


Figure 4.3. Genes that are co-expressed with IL6R and have a reproducible association with asthma or allergies.

Underlined genes were significant at FDR < 5% in the replication analysis. Distance of genes to IL6R, and of eSNPs to genes, represent strength of association (i.e. shorter distances represent stronger association).

Association between STOML2 expression, IL6R expression and asthma risk

To further characterize the association observed between the expression of *STOML2* and that of *IL6R* in the DMAP project, we examined the expression of both genes across the 38 hematopoietic cell types studied. There was a negative correlation between *IL6R* and *STOML2* expression (Figure 4.4); for example, central memory $CD4^+$ T Cells and neutrophilic metamyelocytes had high *IL6R* expression and low *STOML2* expression, whereas megakaryocytes and erythroids had low *IL6R* expression and high *STOML2* expression.

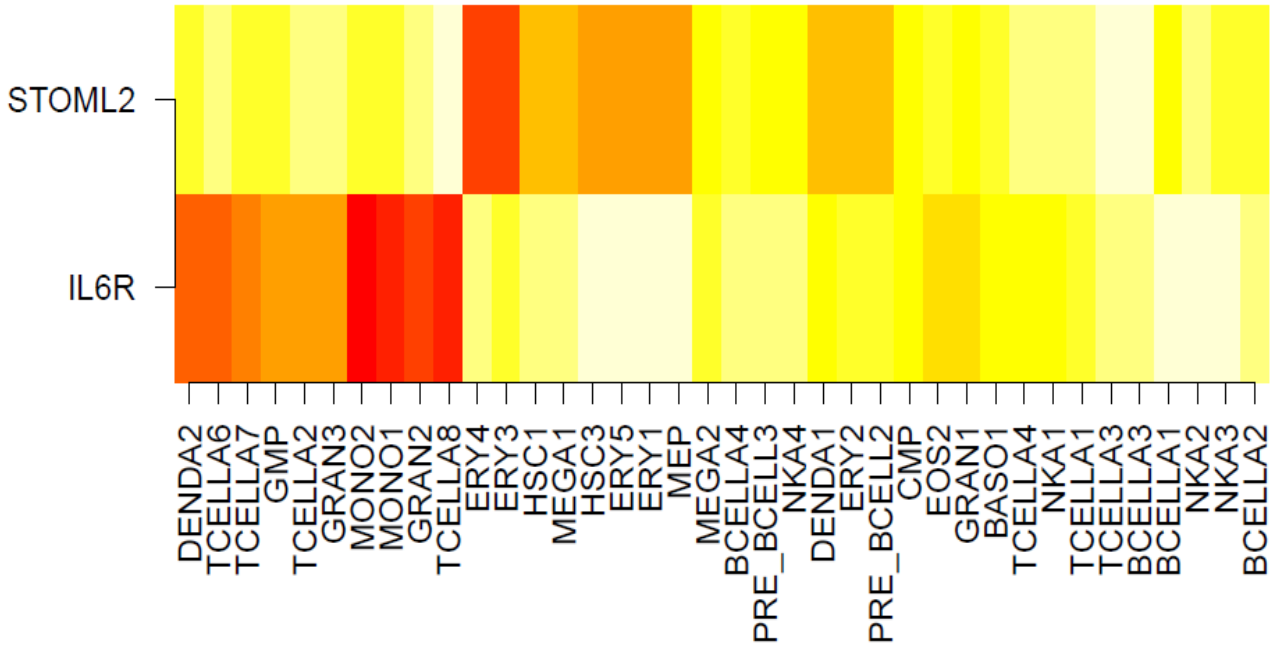


Figure 4.4. Expression of IL6R and STOML2 across 38 hematopoietic cell types.

High gene expression levels are portrayed in red and low gene expression levels in white. Abbreviations: DDENDA2, Myeloid Dendritic Cell; TCELLA6, Naïve CD4+ T Cells; TCELLA7, Effective Memory CD4+ T Cell; GMP, Granulocyte-Monocyte Progenitor; TCELLA2, Naïve CD8+ T Cells; GRAN3, Neutrophil; MONO2, Monocyte; MONO1, Colony Forming Unit-Monocyte; GRAN2, Neutrophilic Metamyelocyte; TCELLA8, Central Memory CD4+ T Cell; ERY4, Erythroid 4; ERY3, Erythroid 3; HSC1, Hematopoietic Stem Cell 1; MEGA1, Colony Forming Unit-Megakaryocytic; HSC3, Hematopoietic Stem Cell 3; ERY5, Erythroid 5; ERY1, Erythroid 1; MEP, Megakaryocyte/Erythroid Progenitor; MEGA2, Megakaryocyte; BCELLA4, Mature B Cell Class Switched; PRE_BCELL3, Pro B Cells; NKA4, NKT; DENDA1, Plasmacytoid Dendritic cell; ERY2, Erythroid 2; PRE_BCELL2, Early B Cells; CMP, Common Myeloid Progenitor; EOS2, Eosinophil; GRAN1, Colony Forming Unit-Granulocyte; BASO1, Basophil; TCELLA4, Central Memory CD8+ T Cell; NKA1, Mature NK Cell 1; TCELLA1, Effective Memory RA CD8+ T Cell; TCELLA3, Effective Memory CD8+ T Cell; BCELLA3, Mature B Cell; BCELLA1, Naïve B Cells; NKA2, Mature NK Cell 2; NKA3, Mature NK Cell 3; BCELLA2, Mature B Cell Class Able To Switch.

We also combined results from the asthma and transcriptome GWAS to predict the direction of effect of *STOML2* expression on asthma risk. The rs7039317:T allele that was associated with increased asthma risk was associated with increased expression of *STOML2* in whole-blood[195] (Table 4.3), suggesting that increased *STOML2* expression has a predisposing effect on asthma.

Asthma association (Ferreira 2014 GWAS)			Gene expression association						
			eSNP in LD with asthma SNP						
SNP rs ID	Effect allele	OR	rs ID	r^2	Gene	P-value	Study	Tissue	Effect on expression*
rs10197862	A	1.24	rs10197862	1.00	*IL18RAP*	$2.8x10^{-137}$	[195]	Whole blood	Decreased
rs10197862	A	1.24	rs950881	0.95	*IL18R1*	0.00025	[195]	Whole blood	Decreased
rs11000805	C	1.07	rs17741873	0.86	*NDST2*	$2.7x10^{-10}$	[195]	Whole blood	Increased
rs11265424	A	0.91	rs6670721	0.81	*SLAMF1*	$3.0x10^{-15}$	[195]	Whole blood	Decreased
rs1253118	T	1.07	rs956901	0.87	*RTN1*	0.00160	[195]	Whole blood	Decreased
rs13212921	T	1.14	rs13217285	0.92	*BTN2A1*	$1.7x10^{-15}$	[181]	LCLs	N/A
rs13416555	C	1.12	rs891058	0.99	*ID2*	$6.1x10^{-08}$	[193]	PBMCs	Increased
rs1464510	A	0.91	rs9864529	0.81	*BCL6*	$5.2x10^{-10}$	[193]	PBMCs	Increased
rs2357792	A	1.08	rs7342715	0.89	*NOD2*	$3.5x10^{-11}$	[193]	PBMCs	Decreased
rs2435206	T	1.08	rs2435211	0.96	*NSF*	$9.4x10^{-05}$	[190]	LCLs	N/A
rs324011	T	1.09	rs12368672	0.89	*STAT6*	$9.8x10^{-198}$	[195]	Whole blood	Decreased
rs4833095	T	1.20	rs12233670	0.97	*TLR1*	$2.8x10^{-57}$	[197]	Whole blood	Increased
rs6511788	T	1.07	rs10411986	0.98	*MAN2B1*	$5.2x10^{-06}$	[181]	LCLs	N/A
rs7039317	T	1.11	rs10972275	0.93	*STOML2*	$9.6x10^{-05}$	[195]	Whole blood	Increased
rs9289837	T	0.88	rs13327359	1.00	*MED12L*	$2.4x10^{-37}$	[195]	Whole blood	Decreased
rs9289837	T	0.88	rs7637803	0.85	*P2RY13*	$2.0x10^{-14}$	[195]	Whole blood	Decreased

Table 4.3. Effect of asthma-associated SNPs on gene expression

** Corresponding to the eSNP allele that is on the same haplotype as the asthma effect allele. Abbreviations: LD, linkage disequilibrium; N/A, not available; OR, odds ratio; SNP, single nucleotide polymorphism*

Other genes near STOML2 regulated by rs7039317

Many eSNPs are known to be associated with the expression of multiple genes, as we observed for rs10197862, an eSNP for both *IL18R1* and *IL18RAP*. Although rs7039317 was only associated with the expression of a single gene that was correlated with *IL6R*, it could nonetheless be associated with the expression of other nearby genes unrelated to the IL-6 signalling pathway, i.e. our results do not exclude the possibility that another gene is the driver of the association between rs7039317 and asthma. When we queried results from 11 published GWAS of gene expression levels, there were an additional three genes whose expression was correlated with this SNP, namely *VCP* ($P = 2 \times 10^{-17}$; [193,195]), *PIGO* ($P = 3 \times 10^{--6}$; [195]) and *DNAJB5* ($P = 6 \times 10^{-4}$; [195]). Therefore, in addition to *STOML2*, these three genes represent putative target genes for rs7039317 and so might also be related to asthma pathophysiology.

Comparison with a random selection of genes

The aim of this study was to identify asthma risk genes amongst *IL6R*-associated genes. This was assessed by identifying eSNPs with a reproducible association with asthma risk amongst eSNPs for *IL6R*-associated genes. We found 14 such eSNPs amongst 61 eSNPs tested (23%), a significant enrichment over the 2.5% expectation (0.05 [$P < 0.05$] x 0.5 [same direction] = 0.025). It is possible that this enrichment of significant asthma associations amongst the eSNPs tested arose mostly because eSNPs in general are more likely to be disease-associated than randomly selected SNPs.[202] To test this, we applied steps A through D (Figure 4.1) to a random gene of interest in place of *IL6R*, and repeated this analysis 1,000 times (see Methods for details). On average, we found that 17.4% (SD = 4.7%) of eSNPs for genes co-expressed with a given random gene had a reproducible association with disease risk, a 1.3-fold decrease when compared to 23% for the list of *IL6R*-associated genes, but this difference was not statistically significant (empirical $P = 0.120$; see Methods for details). The observation that the enrichment of reproducible associations between eSNPs and asthma risk is comparable whether we considered *IL6R* or a random selection of genes does not imply that the associations are false-positives. Instead, it is consistent with previous studies that demonstrate that eSNPs are more likely to be associated with human traits than frequency-matched SNPs that are not related to gene expression.[202] This observation is important to interpret our results but does not detract from the identification of eSNPs that are related to asthma risk as well as the expression of *IL6R*-associated genes.

Discussion

In this study, we investigated the possibility that genes whose expression is associated with that of *IL6R* might also be causally related to asthma pathophysiology.

To identify genes with expression levels associated with that of *IL6R*, we used two complementary approaches, each with its own strengths and weaknesses. The RNA-seq dataset generated by the Geuvadis consortium[181] for 373 unrelated individuals provided a unique opportunity to identify genes that share transcriptional regulatory mechanisms with *IL6R* constitutively in LCLs; for example, these could be shared regulatory elements (e.g. enhancers) or transcription factors. The top three genes most correlated with *IL6R* expression in this analysis were: *FYN* (negative correlation), which regulates mast cell function,[210] B-cell development[211] and is phosphorylated upon IL-6 binding to IL-6R[212]; *CD180* (positive correlation), which belongs to the Toll-like receptor family and is involved in innate immune response to mycobacteria[213]; and *ATP8B2* (positive correlation), which is located

in close proximity (53 kb) to *IL6R*, suggesting that both genes might share a nearby regulatory element. Our results demonstrate that the expression of these genes is to some extent coordinated with that of *IL6R* in LCLs, and is consistent with the previously suggested role for *IL6R* in Treg development,[32] innate immunity[214] and inflammation.[215] The major caveat of this analysis was the use of an immortalized cell line that, although derived from a relevant cell type (B-cells), provides a very limited representation of gene expression patterns that are relevant for asthma.

To partly address the limitation of using LCLs to uncover *IL6R*-associated genes that are relevant for asthma, we also analysed publicly available gene expression patterns measured in 38 different hematopoietic cell populations by the DMAP project.[186] The strengths of this approach included the use of primary cells collected from volunteers (instead of cell lines) and the opportunity to identify genes associated with *IL6R* across cell types within individuals (instead of within a cell type across individuals). As such, the two approaches used (analyses of Geuvadis and DMAP data) shared the same aim but were conceptually distinct. An association between *IL6R* and another gene across cell types within an individual could arise, for example, if differentiation into specific cell lineages from a common progenitor requires temporal coordination of the expression of both genes (e.g. simultaneous expression; expression of one gene but not the other).

In this second approach, the proportion of genes with expression associated with that of *IL6R* (49%) at $P < 0.05$ far exceeded the 5% nominal expectation. However, this is perhaps not too unexpected given that the cell types analysed by the DMAP project not only derive from a common hematopoietic progenitor cell (hematopoietic stem cells $CD133^{+}$ $CD34^{dim}$), but also include cell types that belong to the same lineage: for example, CD8 T cells and CD4 T cells, early B cells and pro B cells. As highlighted in the original DMAP publication,[186] the global transcription profiles from cell types of related lineages are highly correlated.

The top three genes whose expression was most closely associated with that of *IL6R* in the DMAP project were (all with a positive correlation): *CD4*, a cell surface antigen that is expressed on subsets of T cells, as well as on monocytes and macrophages, and plays an important role in T-helper cell development and activation[216]; *VIPR1*, a high-affinity G protein-coupled receptor for vasoactive intestinal peptide, a neuropeptide that controls both innate and adaptive immunity [217,218] and that has long been linked to asthma[219]; and *RGL1*, a downstream effector protein of the Ras pathway of IL-6 signal transduction.

The clear literature links between IL-6 signalling and the function of some of the top *IL6R*-associated genes in the Geuvadis and DMAP analyses, suggests that both approaches were indeed able to identify specific components of the IL-6 signalling transduction pathway, its upstream regulators or downstream target genes. As for *IL6R*, we hypothesized that genetic dysregulation of the expression of these genes could affect asthma risk, for example by interfering with cellular differentiation or function in response to IL-6 stimulation. To address this hypothesis, we identified the subset of *IL6R*-associated genes whose expression was regulated by a SNP that was also a risk factor for asthma in a published GWAS.[188] This list (90 genes) included genes directly relevant for both IL-6 signalling and asthma, for example *TLR1*,[220] *IL17RA*[221,222] and *NDFIP1*,[223] amongst others.

Due to multiple testing, the SNP associations with asthma for some of these 90 genes were likely to represent false-positive findings. To identify those with a reproducible association, we extracted results for these SNPs from an independent asthma GWAS[62] and found that for 16 genes the SNP association with asthma was both significant and consistent. As such, this represents the group of genes which our analyses most convincingly link to both asthma aetiology and IL-6 signalling. Five of these genes were identified at a significance threshold that controls for multiple SNP testing in the replication study (FDR < 0.05): *IL18R1*, *IL18RAP*, *BCL6*, *STAT6* and *STOML2*. The first four, but not *STOML2*, are known target genes of SNPs that have an established (i.e. genome-wide significant) association with allergic disease.[62,208]

IL18R1 and *IL18RAP* encode the alpha and beta chains of the IL-18 receptor; IL-18 signalling through this receptor can activate IL-6 production,[224] and IL-18 serum levels have been found to be positively correlated with sIL-6R levels.[225] Therefore, together with these studies, our results indicate that the IL-18 and IL-6 signalling pathways are associated, and that this could in part be achieved by the coordinated transcription of *IL6R*, *IL18R1* and *IL18RAP*.

BCL6 is a transcription factor with a critical role in the activation and differentiation of germinal centre (GC) B cells[226] and CD4 T cell differentiation into T follicular helper (Tfh) cells.[227] IL-6 stimulation is required for *BCL6* expression, while in turn BCL6 induces *IL6R* expression.[228] Thus, the expression of both genes is associated during specific B and T cell differentiation programs. Our finding that *BCL6* expression is positively correlated with *IL6R* expression across hematopoietic cell types further supports the importance of coordinated transcription of these genes during normal cell lineage commitment. The association between SNPs that regulate the expression of these two genes

and asthma risk suggests that their effect on asthma pathophysiology might be related to dysregulation of GC B-cell and/or Tfh-cell differentiation.

STAT6 is a transcription factor that plays a key role in adaptive immunity, by mediating IL-4 and IL-13 signalling through their cognate receptors,[229] but also in innate immunity.[230] Few studies have reported an association between IL-6 and STAT6, suggesting that these two signalling pathways might be mostly independent. However, in macrophages, IL-6 can induce the expression of the IL-4 receptor and augment IL-4-induced STAT6 signalling.[231] As such, *IL6R* and *STAT6* co-expression might be important for innate immune responses. Consistent with this possibility, in the DMAP project, the cell types with highest expression of both *IL6R* and *STAT6* were monocytes (not shown), the precursors for macrophages.

Lastly, results from our analyses indicate that *STOML2* is a putative novel asthma risk gene whose expression is negatively associated with that of *IL6R*. The overall statistical evidence we found for an association between the *STOML2* eSNP and asthma risk ($P = 2 \times 10^{-6}$) would be considered suggestive and not genome-wide significant in the context of a GWAS. As such, validation of this association in a well-powered independent study is required to unambiguously confirm this eSNP as a novel risk factor for asthma.

STOML2 encodes for a protein that is mostly expressed in mitochondria, is required for the correct development of cell respiratory chain complexes,[232] and promotes T cell activation.[233] In our study, we found that the eSNP associated with increased *STOML2* expression was associated with an increased disease risk. Intuitively, the inverse correlation observed between *STOML2* and *IL6R* expression may seem contradictory to the fact that increased expression of these genes is associated with variants predisposing to higher risk of asthma. Indeed, the rs4129267:T allele was estimated to increased asthma risk by 1.09-fold,[1] and increased serum levels of sIL-6R by 1.4-fold.[27] However, rs4129267:T is in high LD ($r^2 = 0.99$) with rs2228145:C, which was also shown to associate with lower expression of IL6R in plasma and whole blood.[122,234] As discussed by van Dongen et al.,[234] the opposite effect of rs2228145 on sIL-6R protein levels and IL6R expression may be explained by the strong effect that the variant has on IL6R alternative splicing. Specifically, the rs2228145:C is associated with increased splicing of exon 9,[235] which results in a differentially spliced mRNA presumed to directly code for sIL-6R. Consistent with this hypothesis, the rs2228145:C allele have been shown to associate with higher expression of the differentially spliced IL6R mRNA.[119,235]

The association of the *STOML2* eSNP with increased gene expression and increased asthma risk suggests that *STOML2* may have a pro-inflammatory effect in asthma. This is consistent with the observation that T cells from *STOML2*-deficient mice have decreased IL-2 production in response to cell activation, and this translated into reduced CD4+ T cell responses.[236] In the DMAP project, CD4+ T cells had high expression of *IL6R* and low expression of *STOML2*. We speculate that in individuals with the rs7039317:T allele that increases *STOML2* expression,[195] mitochondria biogenesis and function in CD4+ T cells is increased, which results in stronger T cell responses to allergens, thereby increasing asthma risk. Functional studies that formally test this hypothesis are warranted.

Interestingly, IL-6R blockade has been shown to attenuate the development of cachexia by promoting mitochondrial biogenesis and dynamics,[237] which are induced by *STOML2*.[236] These observations are consistent with our finding of a negative correlation between *IL6R* and *STOML2* expression and, collectively, suggest that *STOML2* is part of a gene network that underlies the effect of IL-6 on muscle loss. Of note, skeletal muscle dysfunction is common in chronic obstructive pulmonary disease[238] and is induced by chronic intake of corticosteroids.[239]

In conclusion, we identified 16 genes whose expression was associated with *IL6R* and that were regulated by common polymorphisms that had a reproducible association with asthma risk. This list included five known and 11 putative new asthma risk genes, of which *STOML2* had the strongest SNP association with asthma. These genes provide new clues into broader gene networks that are associated with IL-6 signalling and that contribute to asthma pathophysiology.

Chapter 5

Association between *STOML2* and allergic disease

CHAPTER 5. ASSOCIATION BETWEEN *STOML2* AND ALLERGIC DISEASE

The goals of this chapter were to (1) validate the association reported in the previous chapter between a *STOML2* variant and asthma risk; and (2) to test the association between the same *STOML2* variant and the risk of hay fever and eczema, two allergic diseases that share a large fraction of their genetic make-up with asthma.

Introduction

In the previous chapter, we identified genes associated with *IL6R* that also contributed to asthma risk. To identify genes whose expression correlated with that of *IL6R*, we used data from two publically available gene expression datasets.[181,186] Then, we screened those genes for nearby SNPs associated with both variation in gene expression levels (eSNPs) and asthma risk. We used the Ferreira et al. GWAS,[188] which included cases with both asthma and hay fever, to assess associations with asthma risk. Then, we confirmed those associations using data from the GABRIEL GWAS[62] – the largest asthma GWAS that had been performed at the time the analyses were performed. Amongst the top replicated associations (false discovery rate [FDR] < 0.05) were eSNPs for four known (*IL18R1*, *IL18RAP*, *BCL6* and STAT6) and one putative novel asthma risk gene, stomatin-like protein 2 (*STOML2*).[240] Specifically, *STOML2* expression correlated with that of *IL6R*, and was up-regulated by an allele (rs7039317:T) that was associated with increased asthma risk.

STOML2 encodes a protein found in mitochondria and cell membranes and has previously been shown to play a role in the function of T-cells, which in turn are central to allergic disease. However, even though our results suggest that *STOML2* might contribute to IL-6 signalling in the context of asthma, evidence for association between rs7039317 and asthma risk did not reach the genome-wide significance threshold (combined discovery and replication analysis OR = 1.10 for the T allele, $P = 2 \times 10^{-6}$).[240] Moreover, it was unclear from our results whether *STOML2* was a risk factor that was specific to asthma or potentially shared with other allergic diseases, such as hay fever and eczema. Here, to address these questions, we assessed the association of rs7039317 with multiple allergic disease phenotypes using data from a new study that was not included in the analysis described in Chapter 4: the UK Biobank study.

Methods

Selection of cases and controls for association analysis from the UK Biobank study

The UK Biobank study is a prospective study of 502,682 participants recruited at 22 centres across the UK between 2006 and 2010.[241] Data from this study was accessed through application number 10074. We downloaded data-fields approved as part of this application on the 21st of March 2016, and restricted our analysis to a subset of 152,566 individuals with available genotype data at that time.

We used information from the available data-fields as described in detail previously[242] to classify disease status for asthma, hay fever and eczema. After restricting the analysis to individuals with information available for the SNP of interest near *STOML2* (rs7039317), and who were unrelated to each other (based on an identity by descent cut-off of 0.125), we identified individuals of European ancestry as previously described.[242] Briefly, using 847,442 directly genotyped variants from 152,566 individuals in the UK Biobank, we selected common SNPs (MAF > 5%) in low LD ($r^2 > 0.1$) with each other that were (1) present in the 1000 Genomes Project, with call rate >95% and Hardy-Weinberg equilibrium P value >10-6, and (2) not A/T or C/G polymorphisms. Then, we merged the UK Biobank and 1000 Genomes project data and performed multi-dimensional scaling (MDS) analysis to identify individuals who clustered closely to Europeans of the 1000 Genomes project. Among individuals of confirmed European ancestry, we identified 16,095 cases with asthma, but no hay fever or eczema; 7,130 cases with hay fever, but no asthma or eczema; 2,904 cases with eczema, but no asthma or hay fever; and 86,986 controls who reported not suffering from any allergic disease (see Table 5.1 for demographics of each group). Information from asthma and hay fever was then used to define two new combined phenotypes: "asthma with hay fever", with cases defined as individuals suffering from both diseases (*n* = 2,011); and "asthma and/or hay fever", with cases defined as those who suffered from asthma and/or hay fever (*n* = 21,214).

	Asthma	Hay fever	Eczema	Controls
N	16,095	7,130	2,904	86,986
Sex (% female)	56.6	53.1	56.1	51.4
Age (mean, SD, range)	56, 8.1, 40-70	55, 8.1, 40-70	55, 8.1, 40-70	57, 7.9, 40-73
BMI (mean, SD, range)	28, 5.4, 15-66	27, 4.6, 16-57	27, 5, 17-67	27, 4.7, 14-67
Smoker (% current, % ever)	10.5, 44.3	7.2, 38.1	10.7, 43.6	12.9, 47.7
Age of onset* (mean, SD, range)	30, 18.9, 1-74	24, 15.7, 1-70	23, 19.1, 1-67	-

Table 5.1. Demographics of study participants

**Age of onset for hay fever and eczema was assessed under the same question in the UK Biobank questionnaire. Abbreviations: SD, standard deviation.*

Age-of-onset of allergic disease

Age-of-onset information was obtained from two data-fields: "Age asthma diagnosed" and "Age hay fever, rhinitis or eczema diagnosed". For each individual, the minimum of these two was taken as the earliest any one allergic disease was first diagnosed. We were not able to analyse age-of-onset separately for hay fever and rhinitis, as there were no data-fields covering these two diseases separately. We considered a cut-off of 12 years old to identify cases with early disease onset, because it coincides with the start of the teenage period, which is associated with significant developmental changes (i.e. puberty).

Statistical analyses

We tested the association between allelic dosage of rs7039317 and case-control status using logistic regression. The effects of age, sex, body mass index (BMI), and smoking status (current and ever) on case-control status was tested with a multiple logistic regression beforehand, and variables with significant ($P < 0.05$) effect were included in the model as covariates.

The association between rs7039317 and age-of-onset (quantitative trait) was tested using linear regression, after applying a rank-based inverse-normal transformation. Similarly, age, sex, BMI and smoking status were included in the model as covariates if their effect on age-of-onset was significant ($P <0.05$).

Analyses were performed in R version 3.2.3.

Results

Replication of the association between rs7039317 and asthma with hay fever

We previously found a significant association between rs7039317 near *STOML2* and the combined phenotype of asthma with hay fever (OR = 1.10).[188] Our first goal was to test if a consistent association with this same phenotype was also observed in the UK Biobank study, which was not included in our original analysis. We identified a total of 2,011 cases who reported having both asthma and hay fever, all unrelated and of European descent. On the other hand, a total of 86,986 individuals reported not suffering from any allergic disease and so were considered as controls. In this dataset, we observed a consistent (OR = 1.05 for T allele) but not significant ($P = 0.2036$) association between asthma with hay fever and rs7039317 (Table 5.2).

	N cases	N controls	OR	*P*-value
All asthma with hay fever cases	2,011	86,986	1.05	0.2036
Subset of cases with early onset	849	86,986	1.09	0.1635

Table 5.2. Association results between rs7039317 and asthma with hay fever

Odds ratio (OR) reported for the minor allele (rs7039317:T).

Effect of age-of-onset on the association between rs7039317 and asthma with hay fever

We noticed that the UK Biobank cases included in the analysis above were enriched for individuals with late-onset asthma (e.g. median age-of-onset = 14; 54% with onset > 16), which was not the case in our original study (35% with onset > 16). Age-of-onset of hay fever/eczema was also relatively high (median age-of-onset = 20; 38% with onset > 16). Therefore, we hypothesised that the attenuated effect observed for rs7039317 in the UK Biobank study could reflect stronger effects for this SNP on early- when compared to later-onset allergic disease.

To test this hypothesis, we first tested the association between rs7039317 and age-of-onset of allergic disease. The latter was defined as the earliest age-of-onset reported when considering information from asthma and hay fever/eczema (see Methods), which was available for 34,137 individuals (Figure 5.1). We found that the rs7039317:T allele was significantly associated with decreased age-of-onset (P = 0.0175; Table 5.3). To understand if this effect was similar for asthma as compared to hay fever/eczema, we analysed the two age-of-onset phenotypes separately. We found a significant association with hay fever/eczema (P = 0.0241) but not with asthma (n = 14,014; P = 0.6843) age-of-onset (Table 5.3). This indicates that the significant association with the combined allergic disease age-of-onset phenotype was mostly driven by the association with hay fever/eczema age-of-onset.

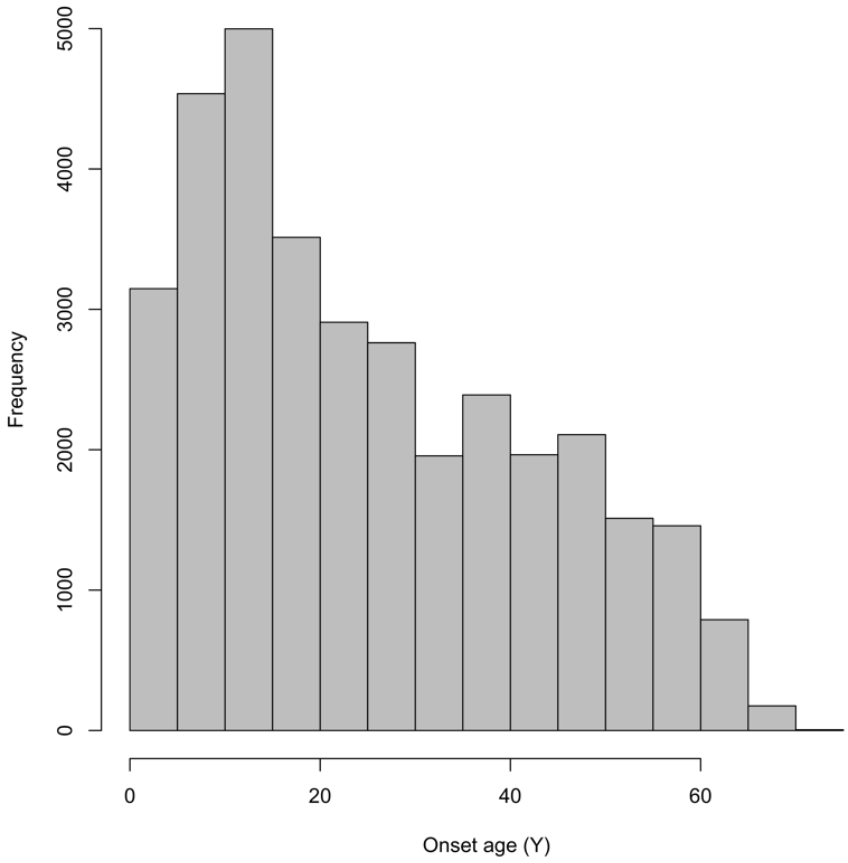


Figure 5.1. Age of onset of allergic disease.

Age-of-onset of:	N	β	*P*-value
Asthma	14,014	-0.01	0.6843
Hay fever/eczema	26,161	-0.03	0.0241
Allergic disease	34,137	-0.02	0.0175

Table 5.3. Association between rs7039317 and age-of-onset of allergic disease.

The results above suggest that rs7039317:T has a stronger effect on disease risk in individuals with early onset of allergic disease. We thus repeated the association analysis between this SNP and asthma with hay fever, but considered only cases with onset of allergic disease before age 12 (n = 849; see Methods for justification of cut-off selection). In this analysis, the effect of rs7039317 on disease risk was stronger (OR = 1.09) and closer to that reported in our original study (OR = 1.10). However, it did not reach statistical significance (P = 0.1635; Figure 5.2 and Table 5.2), a reflection of the smaller sample size.

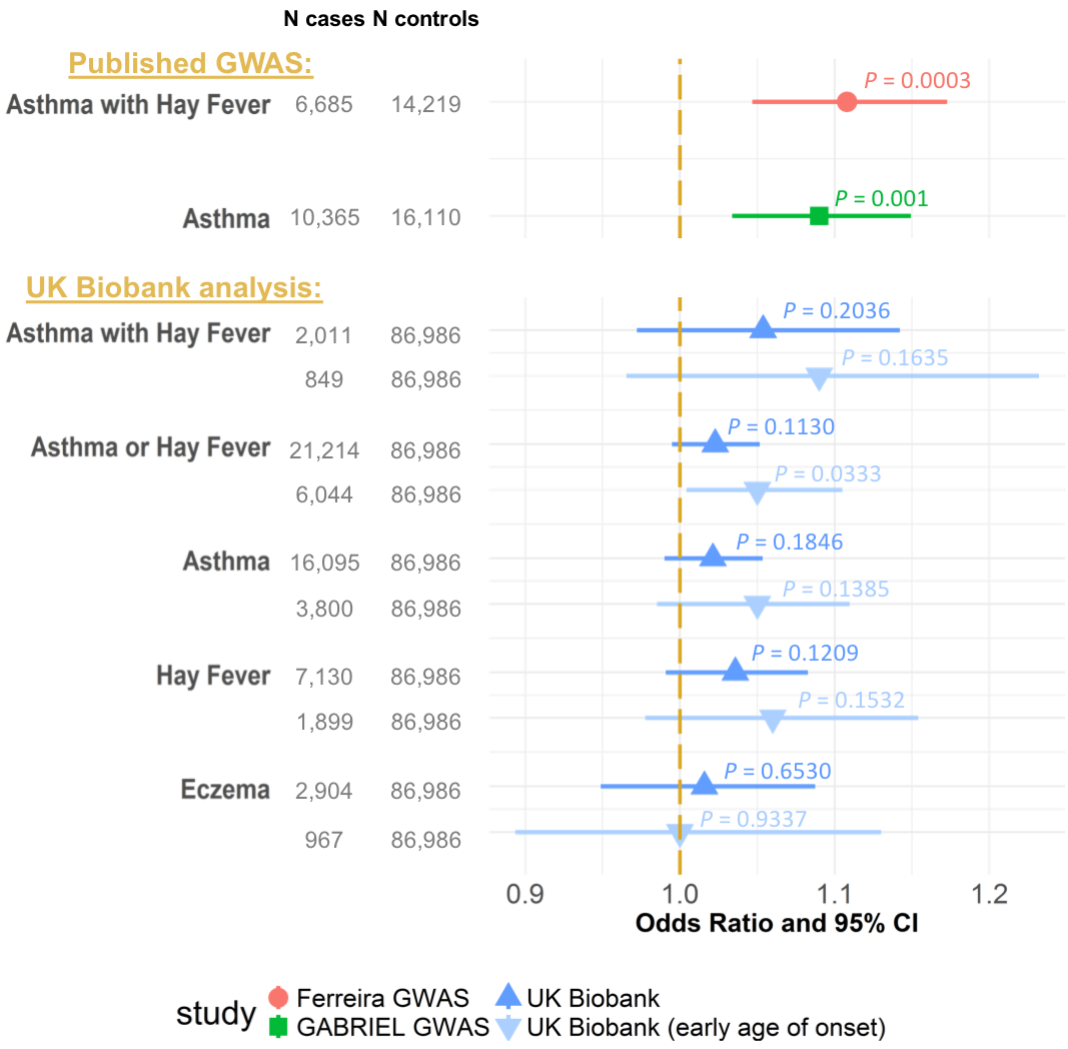


Figure 5.2. Summary of rs7039317:T associations with allergic diseases.

In each study, cases were defined as having the phenotype mentioned on the left. The phenotype "Asthma with Hay Fever" included cases affected by both diseases. The same set of controls was used across all UK Biobank analyses (N = 86,986). Early age-of-onset was defined as between 0-12 years of age. Abbreviations: CI, confidence interval.

Association between rs7039317 and the risk of individual allergic diseases

To help understand if rs7039317 was a genetic risk factor shared between different allergic diseases, we then tested the association between this SNP and case-control status for asthma, hay fever and eczema separately. We found that the rs7039317:T allele was associated with increased risk of hay fever (OR = 1.04), asthma (OR = 1.02) and eczema (OR = 1.02), but all three associations were not statistically significant ($P > 0.05$; Table 5.4). Of note, the SNP effect on the individual risks of hay fever and asthma increased in individuals with early age-of-onset of allergic disease (OR = 1.06 and

OR = 1.05, respectively; Table 5.4), which was not the case for eczema (OR = 1.00). These results suggest that rs7039317 increases the risk of early onset asthma and early onset hay fever similarly, with a weaker or no effect on eczema risk. Consistent with this hypothesis, when we considered cases who reported suffering from asthma or hay fever and had early disease onset (n = 6,044, compared with n = 849 for asthma with hay fever analysis), we found a marginally significant association between rs7039317 and case-control status (OR = 1.05, P = 0.0333). Therefore, we conclude that results from the UK Biobank study do not formally replicate our original association between rs7039317 and asthma with hay fever but generally support the notion that this SNP is a risk factor for both asthma and hay fever with early onset.

	N cases	N controls	OR	*P*-value
Asthma	16,095	86,986	1.02	0.1846
Hay fever	7,130	86,986	1.04	0.1209
Eczema	2,904	86,986	1.02	0.6530
Cases with early onset				
Asthma	3,800	86,986	1.05	0.1385
Hay fever	1,899	86,986	1.06	0.1532
Eczema	967	86,986	1.00	0.9337

Table 5.4. rs7039317 association results with individual allergic phenotypes.

Odds ratio (OR) reported for the minor allele (rs7039317:T). Abbreviations: Y, years.

Discussion

In the previous chapter, we identified a variant near *STOML2* (rs7039317) as a putative novel risk variant for asthma.[240] Specifically, the rs7039317:T allele associated with increased risk of suffering from asthma with hay fever. Here, we studied that association in an independent study to increase confidence in our original results and assessed whether rs7039317 was a risk factor shared between allergic diseases.

We found a weaker, but consistent, association between rs7039317 and the phenotype 'asthma with hay fever'. Of note, the allele that associated with increased disease risk was also associated with earlier age-of-onset, and the association between rs7039317 and 'asthma with hay fever' was stronger in individuals with early age-of-onset. Risk variants associated with early onset disease have previously been reported to be shared between asthma, hay fever and eczema.[242] Our results suggest that rs7039317 is one such variant.

Asthma, hay fever and eczema – also known as the atopic triad – are characterized by IgE-mediated immune responses, which in turn are induced by Th2-type cytokines. Increased expression of *STOML2* has been shown to promote sustained T-cell responses.[233] Thus, we hypothesized that the rs7039317:T allele, which is associated with increased *STOML2* expression,[195] would promote stronger T cell responses and so contribute to an increased risk of multiple allergic diseases. Even though we found no significant associations, we observed that the rs7039317:T allele was associated with a higher risk of each of the three individual diseases, asthma, hay fever and eczema. Of note, stronger associations were observed for both asthma and hay fever in individuals with early-onset disease. This is perhaps not surprising given that genetic burden is higher in individuals with early-onset of these traits. [243] However, the association with eczema was weaker in individuals with early-onset. Eczema often precedes asthma and hay fever,[244] and early-onset has been shown to associate mainly with familial factors, as opposed to environmental exposures.[245-247] In a model of allergic contact dermatitis, *STOML2* was shown to be part of a group genes that are up-regulated by several sensitizers, suggesting its involvement in eczema.[248] Nevertheless, our results suggest that the genetic regulation of this gene is not associated with disease risk.

While the current study did not replicate our original association with asthma with hay fever, the effect size observed in individuals with early-onset was comparable to that reported previously. Together with the weaker association observed for eczema, this observation supports the notion that rs7039317-dependent higher *STOML2* expression increases the risk of early onset asthma and hay fever, that is, allergic diseases of the airways.

Chapter 6

Prevention of allergen-induced asthma exacerbations with tocilizumab

CHAPTER 6. PREVENTION OF ALLERGEN-INDUCED ASTHMA EXACERBATIONS WITH TOCILIZUMAB

The goal of this chapter was to test the hypothesis that asthmatics treated with a single dose of tocilizumab have reduced airway inflammation and bronchoconstriction after allergen challenge.

Introduction

Chapter 1 reviewed extensive clinical, genetic and functional evidence suggesting a pathological contribution of the IL-6 pathway to asthma. Briefly, clinical studies showed that, compared to healthy controls, asthma patients have high systemic[101,249] and airway[75] levels of IL-6, which in turn are associated with impaired lung function[102,111] and worse asthma symptoms.[102,250] Genetic association studies also showed that asthma susceptibility is greater amongst carriers of genetic variants in the *IL6R* gene that associate with increased serum levels of sIL-6R.[1,122] Notably, among these is a genetic variant (rs4129267, or a variant in LD with it) that also associates with the risk of rheumatoid arthritis (RA)[123] – a disease currently treated with anti-IL-6R therapy. Lastly, experiments with mouse models of asthma showed that anti-IL-6R therapy attenuates features of airway allergic inflammation.[32,33,251] Thus, an extensive body of evidence suggests that blockade of the IL-6 pathway may represent a new treatment option for asthma.

Two main therapeutic approaches can be used to inhibit IL-6 signalling, namely targeting IL-6 itself or its receptor. Anti-IL-6 monoclonal antibodies (mAbs) were first reported to drastically increase IL-6 in circulation.[252,253] Specifically, treatment was shown to induce accumulation of plasma monomeric IL-6 complexes that were not eliminated by renal filtration.[252] Since then, improved anti-IL-6 mAbs have been developed,[254,255] and have shown promising results in several stages of clinical testing for inflammatory and autoimmune diseases.[256] Antibodies against IL-6R have also been developed.[257 258-260] Of these, tocilizumab – which blocks both the membrane (mIL-6R) and soluble (sIL-6R) forms of the receptor – was the first drug with demonstrated efficacy to be approved for RA treatment. Currently, it is used in over 100 countries to treat RA and other related inflammatory diseases such as Castleman's disease, and juvenile idiopathic arthritis.[78] Tocilizumab is a promising drug to repurpose for the treatment of other autoimmune and inflammatory diseases with underlying IL-6 signalling dysregulation, such as asthma. Other drugs that block both mIL-6R and sIL-6R are also in clinical development, with sarilumab recently approved in the US and Canada for the treatment of RA.[261] Lastly, an alternative treatment strategy was developed at the University of Kiel to selectively block sIL-6R, inhibiting IL-6 trans-signalling but not classical signalling.[262] This new drug

(recently re-named olamkicpet) completed phase I clinical trials[263] and is now being evaluated in a Crohn's disease phase II study sponsored by i-Mab BioPharma.

IL-6R is therefore a unique example of a target for which clinical development in asthma is supported by multiple lines of evidence (observational clinical studies, genetic association studies and animal studies) and also by the availability of drugs that can be used in phase II trials. Based on these observations, in 2012 Dr Ferreira and Prof Upham obtained funding from the QLD Government to perform a phase II clinical trial of tocilizumab in participants with allergic asthma. Specifically, participants performed an allergen-inhalation challenge test before and after treatment, with endpoints compared between those randomized to a single dose of TCZ or placebo.

Our primary efficacy endpoint was the magnitude of the late asthmatic response (LAR), as this is one of the most clinically relevant outcomes in early efficacy studies.[157] The LAR is characterised by narrowing of the airways – also known as bronchoconstriction, which reflects underlying airway inflammation – that occurs typically 3 to 7 hours after allergen exposure. Allergen-inhalation challenge tests are a widely-used research tool to study the LAR. In these tests, increasing concentrations of allergen are inhaled until an early asthmatic response (EAR) is induced, typically consisting of an FEV_1 fall of 20% from the challenge baseline FEV_1. In approximately 50% of cases, recovery from the EAR is followed by a LAR.[157] However, the prevalence of LAR is also determined by the allergen used in the challenge test. For instance, house dust mite (HDM) and cat allergens are more likely to induce LAR than grass pollen.[264] Interestingly, inhalation of HDM has also been shown to significantly increase sIL-6R serum levels.[31] This suggests that sIL-6R plays a role in bronchoconstriction associated with allergic inflammation. In addition, multiple lines of evidence suggest the involvement of IL-6 signalling in allergen-induced responses, including the LAR. First, allergen exposure has been shown to increase IL-6 signalling in humans, mouse models of asthma, and in vitro models. In humans, allergen inhalation challenge tests have been shown to promote exacerbations with significant increases in serum levels of IL-6 and sIL-6R.[31,101] In mouse models of asthma, allergen inhalation challenge was also shown to increase airway levels of IL-6 and sIL-6R,[32,33,108] and blockade of IL-6 signalling abrogated airway inflammation, a key feature in LAR. In addition, alveolar macrophages isolated from patients who performed a bronchial provocation test and developed a LAR were shown to produce increased levels of IL-6 compared to those isolated from patients who did not develop LAR after allergen challenge.[71] Second, the LAR is characterised by airway inflammation, and both serum and sputum levels of IL-6 have been shown to negatively correlated with FEV1.[102,108,111] Therefore, an inhalation challenge with HDM provides an ideal

approach to test the hypothesis that tocilizumab can prevent bronchoconstriction, as advocated by others.[265]

Here, we evaluated the safety and efficacy of tocilizumab in HDM-induced LAR in 11 patients recruited between September 2014 and July 2017. The original target sample size of the study was 16, allocated 1:1 to placebo and tocilizumab. However, in late 2017, after reviewing blinded results from 11 participants who had completed the trial, the data safety and monitoring board (DSMB) of the study requested an interim analysis to confirm that continuing the trial to completion (i.e. to the target of 16) would be powered to detect a significant effect of tocilizumab on the primary endpoint (LAR), given the observed findings to date. Unblinded results were sent to the chair of the DSMB, who compared the primary endpoint between the two groups and found: (1) no significant differences in the LAR post-treatment; and (2) that, given the observed group means and SD, the target n of 16 would not provide adequate power to detect a significant difference between groups. The trial was therefore stopped for futility and unblinded results released to the research team. In this chapter, I performed preliminary analyses of the safety and efficacy endpoints measured in the trial, which will be expanded in a future publication.

Methods

Study design

We initiated a proof-of-concept phase II clinical trial of tocilizumab in 2012, with the first participant enrolled in September 2014 and the last in July 2017. The aim of this trial was to evaluate the safety and efficacy of a single dose of tocilizumab in 16 patients with mild allergic asthma. The trial used a double-blind, randomized, parallel-group, placebo-controlled design, with participants recruited at three clinical sites: Q-Pharm at the QIMR Berghofer Medical Research Institute (Brisbane, Australia); Princess Alexandra Hospital (Brisbane, Australia); and McMaster University (Hamilton, Canada). The study was divided into three phases (Figure 6.1): (1) screening (visits 1 – 4); (2) treatment (visits 5 – 8); (3) and follow -up (visits 9 – 10).

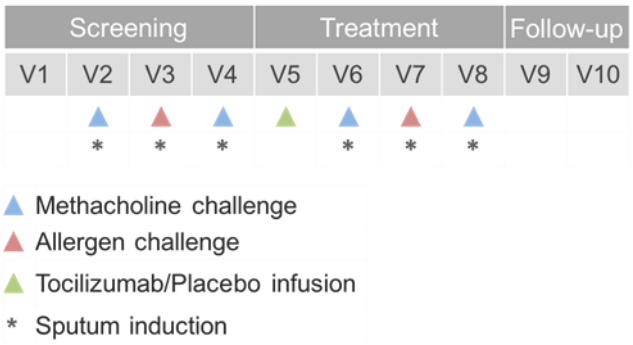


Figure 6.1. Schematic of the 10 clinical trial clinical visits performed in the trial.

We used inhaled-allergen bronchoprovocation tests to assess the efficacy of the drug in preventing allergen-induced airway responses. This acute challenge model is extremely safe, and has been extensively used to evaluate the effect of new anti-inflammatory therapies in asthma.[266]

During the screening phase (visits 1 to 4), participants performed a series of tests to assess the inclusion and exclusion criteria for the study (Table 6.1), which are summarised here and described in detail below. Individuals who remained eligible after the tests performed at visit 1 were mild asthmatics (but otherwise in good health), with confirmed positive skin prick test for HDM and with rs4129267:TT or CT genotype. These participants then progressed to the first allergen inhalation challenge triad (visits 2 to 4), performed within 20 days of visit 1. In the first day of the triad (visit 2), participants completed a methacholine inhalation test and a skin prick titration test (described in detail below). For participants with a positive methacholine test, information from both tests (methacholine and skin titration) was used to determine the allergen PC_{20} as described previously.[160] The allergen PC_{20} corresponds to the allergen provocative concentration required to induce a 20% fall in FEV_1. In the second day of the allergen triad (visit 3), an allergen inhalation challenge test was performed with HDM to determine the presence of an EAR and LAR. In the final day of the triad (visit 4), a follow-up methacholine challenge test was performed. Subjects with confirmed allergen-induced EAR and LAR at visit 4 were eligible to enter the second phase of the study.

Visit	Test
Visit 1	Written informed consent
	Demographics, medical and medication histories
	Physical examination and vital signs
	Spirometry
	Chest X-ray (can be completed any time prior to starting visit 2 testing)
	Resting 12-lead electrocardiogram (ECG)

	Urine and serum pregnancy test for women of childbearing potential
	Participants who continue to meet entry criteria:
	Complete routine laboratory tests;
	Complete skin prick testing against house dust mite.
	Genotype rs4129267 (or any time prior to Visit 1 if required)
Visit 2	Spirometry
	Methacholine challenge test
	Participants who continue to meet entry criteria will:
	Undergo skin titration test
	Sputum induction
Visit 3	Spirometry
	Allergen inhalation challenge test
	Sputum induction
	Blood samples for haematology, biochemistry and biomarker assessment
Visit 4	Spirometry
	Methacholine challenge test
	Sputum induction

Table 6.1. List of tests performed in the screening phase to assess eligibility for the study.

In the second phase of the study (visits 5 to 8), participants were randomized to receive a single dose of either tocilizumab or placebo (visit 5) and performed a second allergen inhalation challenge triad (visits 6 to 8), as summarised above and described in more detail below. The procedures used for randomisation and drug administration are described in detail below. Tocilizumab (or placebo) was administered seven days after the first allergen challenge, and seven days before the second allergen challenge. The timing of drug administration was decided based on the dose-dependent half-life of tocilizumab. Specifically, in RA, an 8 mg/kg dose of tocilizumab administered every four weeks has an apparent half-life of up to 13 days.[267] Thus, in our study, we administered tocilizumab six to eight days before second allergen challenge. In turn, second allergen challenge was performed 14 days after the first allergen challenge, as this is the minimum recovery time shown to be successful in parallel-group studies involving multiple allergen challenges.[157] Drug efficacy was assessed based on primary and secondary endpoints (described in detail below) measured during this second allergen challenge.

Lastly, two follow-up visits were carried out to assess safety endpoints, specifically 14 (visit 9) and 28 (visit 10) days after tocilizumab/placebo infusion, in accordance with the published half-life of tocilizumab.[268]

Procedure used for participant recruitment

Information from participants of asthma studies conducted prior to this trial at the QIMR Berghofer Medical Research Institute and the McMaster University were screened to identify those who were potentially eligible according to inclusion and exclusion criteria of the screening phase of this study (Table 6.2). Potential eligible participants identified were approached by email or postal letter to provide information about this trial, explain why they were considered to be eligible and invite them to participate. In parallel, the study was also advertised in the general community to identify additional potential eligible participants.

Inclusion criteria for entry into screening phase of the study

1. Male and female volunteers 18 through 65 years of age
2. Females must not be actively seeking pregnancy and demonstrate a negative serum pregnancy test at screening
3. Females of child-bearing potential and males with female partners of child-bearing potential must use adequate and effective contraception
4. General good health
5. History of, or current mild to moderate, stable, allergic asthma
6. History of episodic wheeze and shortness of breath
7. FEV_1 at baseline at least 70% of the predicted value
8. Positive skin-prick test to house dust mite
9. Able to understand and give written informed consent and has signed a written informed consent form approved by the investigator's HREC

Inclusion criteria for entry into treatment phase of the study

1. Positive methacholine challenge
2. Positive allergen-induced early and late airway bronchoconstriction
3. rs4129267 genotype TT or CT

Exclusion criteria for both screening and treatment phases of the study

General:

1. History or symptoms of clinically significant autoimmune disease
2. Lung disease other than mild to moderate allergic asthma
3. Recent (less than 1 year) history of alcohol dependency
4. Use of tobacco products of any kind currently or within the previous 12 months, or smoking history > 10 pack years
5. Unwillingness or inability to comply with the study protocol for any other reason

Exclusions because of previous or concomitant therapy:

1. Use of corticosteroids, immunosuppressives, anticoagulants (warfarin or heparin) or any medications that may interact with drug within 28 days prior to treatment randomisation
2. Have chronic use of any other medication for treatment of allergic lung disease other than short- and intermediate-acting ß2-agonists or ipratropium bromide
3. Participation in any other investigational drug treatment protocol within the preceding 30 days or 5 half-lives of the drug

Exclusions for general safety:

1. A worsening of asthma or a respiratory tract infection within 6 weeks preceding study entry
2. History of clinically significant hypotensive episodes or symptoms of fainting, dizziness, or light-headedness
3. History or symptoms of cardiovascular disease, particularly coronary artery disease, arrhythmias, hypertension, or congestive heart failure
4. History or symptoms of significant neurologic disease, including transient ischemic attack (TIA), stroke, seizure disorder, or behavioural disturbances
5. History of serious adverse reaction or hypersensitivity to any drug
6. Abnormal chest X-ray, including evidence of latent or active TB
7. Abnormal electrocardiogram
8. History of clinically significant hematologic abnormality, including coagulopathy
9. History of intestinal ulceration or diverticulitis
10. Clinically significant abnormalities in laboratory test results (including complete blood count, coagulation, chemistry panel and urinalysis)
11. Be pregnant or lactating or have positive serum pregnancy test at screening or positive urine pregnancy test during the study
12. History of, or known currently active, recurrent bacterial, viral, fungal, mycobacterial or other infections (including but not limited to tuberculosis and atypical mycobacterial disease, hepatitis B and C, and herpes zoster, but excluding fungal infections of nail beds), or any major episode of infection requiring hospitalisation or treatment with intravenous antibiotics within 4 weeks of screening or oral antibiotics within 2 weeks prior to screening
13. Primary or secondary immunodeficiency (history of or currently active)
14. Patients with lack of peripheral venous access
15. Concomitant disease or condition which could interfere with the conduct of the study, or for which the treatment might interfere with the conduct of the study, or which would, in the opinion of the investigator, pose an unacceptable risk to the subject in this study, including, but not limited to, cancer, alcoholism, drug dependency or abuse, or psychiatric disease
16. Subject develops a severe asthma reaction during the screening allergen challenge that could not be rescued by bronchodilator alone

Laboratory exclusion criteria (at screening):

1. Serum aspartate transaminase or alanine transaminase >1.5x ULN
2. Absolute neutrophil count < 2 x 10^9/l
3. Platelet count < 100 x 10^9/l

Table 6.2. Eligibility criteria for the study.

Participants interested in taking part in the trial were contacted by phone to confirm interest in participating and to assess potential eligibility based on inclusion criteria for entry into the screening phase of the study (listed in Table 6.2**)**. An appointment for clinical visit 1 was made for participants who remained potentially eligible to participate.

In some cases, to facilitate recruitment some participants had their rs4129267 genotype determined prior to trial enrolment. Participants provided a 4 ml blood sample for genotyping, and completed a separate Participant Information Sheet and Consent form for this collection only.

Prior to performing any study-specific procedures (including screening procedures to determine eligibility), a signed consent form was obtained for each subject. The consent form described the purpose of the study, the procedures to be followed, and the risks and benefits of participation. The investigator conducted the informed consent discussion and checked that the subject comprehended the information provided and answered any questions about the study. Consent was voluntary and free from coercion. The investigator who conducted the consent discussion also signed the informed consent form. A copy of the consent form was given to the subject, and the outcome of the procedure documented. When all the inclusion and exclusion criteria were assessed and eligibility confirmed, subjects were randomised to treatment (phase 2).

Summary of participant inclusion and exclusion criteria

All inclusion and exclusion criteria assessed in the study are listed in Table 6.2. Briefly, non-smoking volunteers, in general good health, aged 18 to 65, with a history of episodic wheeze and shortness of breath were screened for participation in visit 1.

To ensure the safety of the patients and minimise the risk of severe bronchoconstriction during the allergen inhalation challenge test, subjects screened in visit 1 were also required to have stable, mild asthma, and a baseline forced expiratory volume in 1 second (FEV_1) of at least 70% of the predicted value. Patients were also required to have allergic asthma and a positive skin-prick test to HDM because of the model (allergen inhalation challenge test) that we used to test the efficacy of the drug.

Patients enrolled in the study were required to have a positive methacholine challenge ($\geq$ 20% FEV_1 drop). This was essential because the methacholine concentration at which FEV_1 drops 20% (methacholine PC_{20}) is required to determine the allergen PC_{20}.

In the screening phase, patients who did not develop an allergen-induced EAR before the maximum allergen concentration was reached were excluded from the study. Similarly, patients who did not develop a LAR in the screening phase were excluded from the study, as the magnitude of the LAR was the primary efficacy outcome assessed in the study (described in detail below).

Another requirement for inclusion in the study was that subjects were carriers of the rs4129267:T allele. Mounting evidence, including observations from genotype-specific studies, shows that different asthma subtypes have variable response to treatment, and support the notion of developing personalised treatment approaches to manage asthma.[269,270] In our study, the decision to study the rs4129267:CT and rs4129267:TT genotypes was based on prior evidence that tocilizumab response to treatment is associated with polymorphisms in the *IL6R* gene.[271] In addition, several lines of evidence suggest that patients with airway inflammation associated with IL-6 trans-signalling are more likely to benefit from anti-IL-6R treatment. The anti-inflammatory effects of blocking IL-6 trans-signalling in asthma were demonstrated in functional studies with mouse models of asthma.[32,33,215] The rs4129267:T asthma risk allele[1] was estimated to increase serum sIL-6R (the main player in IL-6 trans-signalling) by 1.4-fold,[27] and is in high LD (r^2 = 0.99) with the rs2228145:C functional variant that promotes sIL-6R production. Accordingly, in this proof-of-concept study, we opted not to include patients with the rs412927:CC genotype.

Subjects were excluded from the study if they experienced worsening of asthma, or a respiratory tract infection, within 6 weeks of study entry. Other key exclusion criteria included history or symptoms of autoimmune disease, lung disease (other than mild to moderate allergic asthma), and use of corticosteroids, immunosuppressives, anticoagulants or any medications that could interact with tocilizumab within 28 days of treatment randomization.

Clinical procedures

Medical history

Information collected included, but it is not limited to, current and previous illnesses, hospital admissions, known allergies, history of anaphylaxis, participation in other investigational drug treatment protocols, use of tobacco products and recent (<1 year) history of alcohol consumption.

Physical examination (including X-ray)

The following parameters were measured: height, weight, heart rate, respiratory rate, blood pressure, temperature, examination of body systems, including a resting 12-lead electrocardiogram, antero-posterior chest X-ray and collection of urine sample for analysis.

Lung function

A standard spirometer that complies with 1995 ATS requirements was used for lung function measurements (MicroLab spirometer), with the subject tested while sitting. The spirometer records a

trace of volume against time or volume against flow. All tracings were recorded by the spirometer's software. Results were also entered on the clinical form of each participant.

Skin prick test

Two skin prick tests were performed, namely (1) a skin-prick challenge test, and (2) a skin-prick titration test. Briefly, the skin-prick challenge test was used to identify an allergic reaction to the house dust mite *Dermatophagoides farinae*. The allergen, positive (histamine) and negative controls were purchased from an accredited provider (HollisterStier or Greer). The test was then performed by applying serial dilutions of the house dust mite allergen extract to the skin (from 1:4 to 1:32768) scratching or pricking the skin to allow exposure. The size of the resulting skin wheal was measured 15 minutes after (or 10 minutes after for histamine). The skin-prick titration test was used to identify the lowest titration of allergen causing a skin wheal of at least 2 x 2 mm in size.

Methacholine inhalation challenge test

Briefly, the goals of this test were three-fold: (1) determine the presence of airway hyperresponsiveness, consistent with the diagnosis of asthma; (2) establish the severity of airway hyperresponsiveness as an indirect measure of the likely severity of clinical asthma; and (3) determine the methacholine PC_{20} which is one of two parameters used for predicting the allergen PC_{20}. The method consists of continuous generation of methacholine aerosols at different concentrations, as described by Cockcroft et al.[272] Each concentration of methacholine was then inhaled by the subject using tidal breathing from a Wright nebulizer (Roxon Meditech, Montreal, PQ, Canada) for a duration of 2 minutes. This specific nebulizer is unregistered in Australia but has been extensively used in research studies and clinical trials by our collaborators Prof Paul O'Byrne and A/Prof Gail Gauvreau (McMaster University, Canada) in the last 30 years;[272] its performance is very well characterised, being very precise, reliable and safe. FEV_1 was measured after each inhaled concentration until a 20% fall was observed. Results were expressed as a PC_{20}, representing the provocation concentration of methacholine that causes a fall in FEV_1 of 20%.

Allergen inhalation challenge test

An allergen inhalation challenge test was performed before (visit 3) and after (visit 7) treatment. Briefly, the goal of this test was to elicit an inflammatory response in the airways similar to that which follows natural exposure to allergen. The test includes measuring: (1) the magnitude of the EAR, that is, the largest fall in FEV_1 that occurs 0-3 hours following allergen inhalation; (2) the magnitude of

the LAR, that is, the largest fall in FEV_1 that occurs at 3 - 7 hours; (3) and airway inflammation, based on cell counts and inflammatory mediators measured in induced sputum. House dust mite allergen was inhaled by each subject using tidal breathing from a Wright nebulizer, as described by O'Byrne et al.[273] The inhalation test was performed in a designated area, with emergency equipment and medical facilities readily available. The test was carried out by qualified study personnel under physician supervision, i.e. an investigator or delegate aware of the procedure was present in the laboratory or was available by phone/pager within minutes. Participants were clinically monitored with regular spirometry performed throughout the test. FEV_1 was recorded at regular intervals up to 7 hours post allergen inhalation. Participants who experienced a positive EAR (largest fall in FEV1 >20%) and a positive LAR (largest fall in FEV1 >15%) were eligible to continue to the treatment phase of the trial

Sputum induction and processing

Briefly, the goal of this test was to collect an induced sputum sample for accurate non- invasive assessment of bronchial inflammation. A saline solution was inhaled for increasing concentrations from a DeVilbiss ultrasonic nebulizer (DeVilbiss Healthcare, Castle Hill, NSW, Australia). This nebulizer is currently used by many respiratory function laboratories across Australia, including at the Princess Alexandra Hospital. FEV_1 was measured 60 sec after each saline dose, after which participants were asked to expectorate into a sterile container. Sputum was induced after methacholine (visits 2, 4, 6 and 8) and allergen (visits 3 and 7) inhalation challenges (Figure 6.1). Sputum subtypes were defined as described before, i.e. eosinophilic (≥ 1% eosinophils), neutrophilic (≥ 61% neutrophils), mixed granulocytic (≥ 1% eosinophils and ≥ 61% neutrophils) and paucigranulocytic (< 1% and < 61% neutrophils).

Blood collection and processing

Up to 40 ml of blood were collected at each visit for haematology, biochemistry, immune, inflammatory, genetic and/or pharmacodynamics analyses. Biochemistry analyses were performed with samples collected at visits 1, 3, 5, 7, 9, and 10. In visits where a methacholine or an allergen inhalation challenge test was performed (visits 2-4 and 6-8), blood was collected after the challenge.

Measurement of cytokines in serum and sputum samples

Enzyme-linked immunosorbent assays (ELISAs) and Cytometric Bead Arrays (CBAs) were used to measure inflammatory mediators in serum and sputum supernatant samples. Measurements were

performed according to the kit protocols. CBAs were performed with Becton Dickinson Biosciences kits (Hu Solbl Ptein CBA Buf Kit 100Tst, 558264; Hu TNF CBA Flex Set C4 100Tst, 560112; Hu IL-13 CBA Flex Set E6 100Tst, 558450; Hu IL-8 CBA Flex Set A9 100Tst, 558277; Hu IL-6 CBA Flex Set A7 100Tst, 558276; Hu IL-5 CBA Flex Set A6 100Tst, 558278). ELISAs were performed with Invitrogen (Neutrophil elastase human ELISA kit, BMS269) and R&D Systems (R&D Systems Human IL-6 R alpha Quantikine SixPak (6 Plates), Per Pack, RDSSR600; R&D Systems Human C-Reactive Protein/CRP Quantikine ELISA Kit, Per Kit, RDSDCRP00) kits.

DNA sequencing to obtain rs4129267genotype

DNA was extracted from buffy coats, and the DNA region surrounding rs4129267 amplified and analysed using Sanger Sequencing to obtain genotype data for this SNP. Sequencing was performed by the Australian Genome Research Facility (AGRF), a facility accredited by the National Association of Testing Authorities, Australia (NATA).

Procedure used to randomise participants to tocilizumab or placebo treatment

A block randomisation method was used to allocate the study drug or placebo to the study participants. Briefly, we first randomised tocilizumab or placebo (50:50) to an investigational drug kit (numbered 1 to 16). Then each participant was randomly allocated a drug kit. This procedure was done independently in four blocks with four participants each. The resulting randomisation table was held by the DSMB Secretariat throughout the duration of the study. Based on the randomisation table, two envelopes were created for each participant and sent to the PAH or Q-Pharm pharmacy. Both envelopes contained the same information, namely the participant number, the block number, the kit number and the kit content. Envelope 1 was labelled with the project name, project number, participant number and with "Pharmacy Randomisation code" and "To be opened by trial pharmacist only to prepare the investigational drug". Envelope 2 was labelled with the project name, project number, participant number and with "Emergency unblinding envelope" and "To be opened by investigator only in an emergency situation". 1-2 days prior to visit 5 a study doctor completed a script requesting an investigational drug kit for the participant. The participant's weight was included in this request, so that the right dose of drug could be prepared. The investigator collected the investigational drug kit for the participant, together with envelope 2, which remained closed for the duration of the study. Envelope 1 was kept at the PAH or Q-Pharm pharmacy until the conclusion of the study. A similar procedure was used at McMaster University.

Infusion of tocilizumab or placebo

Tocilizumab (trade name Actemra) and placebo were acquired from, prepared and labelled by, the PAH or Q- Pharm pharmacy. At the PAH this procedure was supervised by the PAH Clinical Trial pharmacist at Q-Pharm this procedure was supervised by the Q-Pharm pharmacist. Tocilizumab (at a dose of 8mg/kg – concentration approved for RA treatment[274]) or placebo were administered at room temperature by controlled infusion into an arm vein over a one-hour period on visit 5. A sterile saline solution, with appearance identical to that of the drug, was used as placebo.

Primary and secondary endpoints

The primary outcomes used to evaluate tocilizumab safety were haematology and blood biochemistry results. The primary outcomes used to evaluate tocilizumab efficacy were (1) the maximum percentage fall in FEV_1 measured during the LAR induced by allergen challenge (LAR $\%fall_{max}$); and (2) the area under the FEV_1 curve during allergen-induced LAR ($AUC_{3\text{-}7h}$).

The calculate the LAR $\%fall_{max}$, for each participant, we (1) identified the largest drop in FEV_1 observed 3 to 7 hours after allergen challenge at visit 3 (LAR $\%fall_{max,screening}$); (2) identified the largest drop in FEV_1 observed 3 to 7 hours after allergen challenge at visit 7 (after treatment; LAR $\%fall_{max,treatment}$); and (3) calculated the difference between the two as LAR $\%fall_{max}$ = LAR $\%fall_{max,treatment}$ - LAR $\%fall_{max,screening}$. For example, in the first participant to complete the trial, LAR $\%fall_{max,screening}$ = 16% (i.e. FEV_1 dropped by 16% from baseline), LAR $\%fall_{max,treatment}$ = 25%, and so LAR $\%fall_{max}$ = 8.75%. In this participant, allergen-induced bronchoconstriction was higher after treatment than at screening. The same approach was used to calculate the $AUC_{3\text{-}7h}$. Specifically, for each participant we (1) calculated the area under the FEV_1 (expressed as % from baseline) curve measured 3 to 7h after allergen challenge at visit 3 ($AUC_{3\text{-}7h,screening}$); (2) calculated the area under the FEV_1 curve measured 3 to 7h after allergen challenge at visit 7 ($AUC_{3\text{-}7h,treatment}$); and (3) calculated the difference between the two as $AUC_{3\text{-}7h}$ = $AUC_{3\text{-}7h,treatment}$ - $AUC_{3\text{-}7h,screening}$. AUC were calculated using the Bolstad2 R package.[275]

The secondary outcomes used to evaluate tocilizumab efficacy were (1) the lowest FEV_1 measured during the EAR induced by allergen challenge (EAR $\%fall_{max}$); (2) airway hyperresponsiveness (i.e. the methacholine PC_{20}) measured 24 hours after the allergen inhalation test; (3) changes in sputum inflammatory cells at 7 and 24 hours after the allergen inhalation test; and (4) serum and sputum inflammatory mediators at 7 and 24 hours after the allergen inhalation test.

EAR $\%fall_{max}$ was calculated for each participant in the same way as LAR $\%fall_{max}$, but with FEV_1 measured 0 to 3 hours after allergen challenge, i.e. EAR $\%fall_{max}$ = EAR $\%fall_{max,treatment}$ - EAR $\%fall_{max,screening}$. Similarly, $AUC_{0\text{-}3h}$ was defined as $AUC_{0\text{-}3h} = AUC_{0\text{-}3h,treatment} - AUC_{0\text{-}3h,screening}$.

Determination of target sample size

Based on the mean (23.5%) and standard deviation (3.3%) of the maximum percentage fall in LAR FEV_1 expected in placebo,[276] we estimated that 16 participants would be needed to detect a difference in LAR between treatment groups of at least 5% (e.g. 83.5% vs. 92.5%).

Statistical analyses

The primary and secondary outcomes were compared between (within) treatment groups with unpaired (paired) Wilcoxon tests. Sputum samples with low viability (< 40%) were removed from analysis of sputum cell counts. Analyses were performed with R version 3.3.2.

Medical monitor, data safety and monitoring board (DSMB) and study monitoring

The medical monitor for this trial was Prof. Ian Yang (Prince Charles Hospital, Brisbane). The DSMB was composed of Prof. Peter Sly (chair; Child Health Research Centre, University of Queensland, Brisbane), Prof. Peter Nash (Department of Medicine, University of Queensland, Brisbane), Prof. Daman Langguth (Wesley Hospital, Brisbane) and Dr. Paul Griffin (Q-Pharm, Brisbane). Adherence to Good Clinical Practice guidelines was monitored by Clinical Network Services (CNS) Pty Ltd.

Interim analysis

In August 2017, after reviewing blinded results from 11 participants who had completed the trial at that time point, the data safety and monitoring board (DSMB) of the study requested an interim analysis to confirm that continuing the trial to completion (i.e. to the target of 16) would be powered to detect a significant effect of tocilizumab on the primary endpoint, given the observed findings to date. Unblinded results were sent to the chair of the DSMB, who compared the primary endpoint between tocilizumab and placebo.

Ethics statement

All study procedures were approved by the ethics committees of the QIMR Berghofer Medical Research Institute (project P2025 and P2103), Metro South (HREC/14/QPAH/22 - SSA/14/QPAH/216) and McMaster University (project 14-790) and carried out according to the Declaration of Helsinki, the NHMRC National Statement on Ethical Conduct in Research Involving

Humans (1999) and the Notes for Guidance on Good Clinical Practice as adopted by the Australian Therapeutic Goods Administration (2000) (CPMP/ICH/135/95) and the ICH GCP Guidelines. All participants provided written informed consent before enrolling in the study. The trial was registered in the Australian New Zealand Clinical Trials Registry (ANZCTR), number ACTRN12614000123640.

Results

Recruitment rate

Over 3,500 individuals were invited through social media or email to be screened at the Brisbane sites to participate in the trial. A total of 607 people were interested in the study, with 446 being screened by phone or email to assess potential eligibility. Of these, 44 were considered potentially eligible and attended visit 1. At McMaster, participants from previous studies were screened to identify 22 potentially eligible participants.

Number of participants who enrolled the study prior to the interim analysis

Across the Brisbane and McMaster sites, a total of 66 participants consented to enrol in this study and completed visit 1 between September 2014 and July 2017 (Table 6.3): 10 at QIMR Berghofer, 34 at the Princess Alexandra Hospital and 22 at McMaster University. Of these, 37 remained eligible for the trial after the assessments performed at visit 1. The most frequent causes of ineligibility were not having the required rs4129267 genotype (n = 20), and having a negative skin prick test to HDM (n = 7). Eight participants withdrew from the study after visit 1, while 29 completed visit 2. Of the latter, most (21 or 72%) went on to complete visits 3 and 4, that is, the full pre-treatment allergen inhalation challenge triad (visits 2 to 4). Of these, 9 were ineligible to continue, most (8 or 89%) because of a negative LAR after allergen challenge. Of the 12 participants who were eligible to continue into the treatment phase of the trial, 11 completed visit 5 (six treated with tocilizumab, five with placebo) and all five subsequent trial visits.

Study visit	QIMR Berghofer	PA Hospital	McMaster University	Total
1	10	34	22	66
2	6	15	8	29
3	4	11	6	21
4	1	11	6	18
5-10	1	4	6	11

Table 6.3. Number of participants enrolled in the study in each study visit.

Abbreviations: PA, Princess Alexandra.

Table 6.4 summarizes demographics and relevant inflammatory features observed before treatment for these 11 participants. Seven patients took Ventolin in the six months prior to entering the study ($n_{Placebo} = 4$; $n_{Tocilizumab} = 3$). No other asthma medications were taken by any of the study participants before enrolment.

	Placebo	Tocilizumab
N	5	6
Age, years	29 (19-51)	35 (25-44)
Sex, % female	80	67
BMI, Kg/m^2	25.5 (23-32)	25 (22-31)
$FEV_{1,}$ % Predicted	92.2 (87-100)	90.7 (72-101)
EAR, % from baseline	69.1 (57-80)	68.3 (59-79)
LAR % from baseline	74.6 (71-81)	78.1 (69-84)
Methacholine PC_{20} pre-Ag, mg/ml	2.9 (0.56-5.74)	6.1 (0.44-14.45)
Methacholine PC_{20} post-Ag, mg/ml	1.1 (0.21-2.15)	2.2 (0.04-8.65)
Sputum sIL-6R, pg/ml	45.8 (0-110.8)	306.3 (0-571.3)
Sputum IL-6, pg/ml	5 (0-11.8)	33.5 (0-75.9)
Serum sIL-6R, ng/ml	97.8 (58.9-149.1)	150.8 (102.9-203.9)
Serum IL-6, pg/ml	1.8 (0-7.5)	1.7 (0-10)

Table 6.4. Patient demographics and inflammatory features in screening phase.

Results are presented as mean and range, unless otherwise stated. Cytokine levels refer to visit 2, i.e. after the pre-allergen methacholine challenge. There were no significant ($P < 0.05$) differences between the groups. Abbreviations: Ag, allergen; BMI, body mass index; FEV_1, forced expiratory volume in 1 second; PC_{20}, provocative concentration causing a 20% decrease in FEV_1.

Interim analysis

An interim analysis was carried out to assess whether continuing the trial to completion (i.e. to the target of 16) would be powered to detect a significant effect of tocilizumab on the primary endpoint, given the observed findings to date. Results from the 11 participants were analysed by the chair of the DSMB (Table 6.5) who compared the primary endpoint between tocilizumab and placebo. The analysis showed (1) no significant differences in the LAR post-treatment; and (2) that, given the observed group means and SD, the target sample size of 16 would not provide adequate power to detect a significant difference between groups. The trial was therefore stopped for futility and unblinded results released to the research team for analysis.

		Screening		Treatment			
Subject ID	Treatment group	$FEV_{1,baseline}$	LAR	$FEV_{1,baseline}$	LAR	LAR $\%fall_{max}$	$AUC_{3\text{-}7h}$
P210300001	Placebo	3.07	2.25	3.07	2.57	-10.42	16.9381
P210300002	Tocilizumab	2.97	2.06	3.25	1.41	25.98	-94.0752

P210300003	Placebo	3.7	2.69	3.65	1.87	4.48	-34.02
P210300004	Placebo	3.53	2.65	3.46	2.16	12.64	-18.1901
P210300005	Tocilizumab	2.39	2.01	2.28	1.67	10.85	-37.0614
P210300006	Tocilizumab	4.15	3.17	4.14	3.59	-10.32	18.6415
QPS0100	Tocilizumab	4.47	3.4	4.45	3.23	3.48	-5.69
S00013	Tocilizumab	3.17	2.67	3.1	2.34	8.75	-34.525
S00023	Tocilizumab	2.98	2.34	2.98	2.37	-1.01	3.5235
S00028	Placebo	3.44	2.45	3.71	2.37	7.34	-51.4178
S00030	Placebo	3.41	2.76	3.4	2.45	8.88	-12.22

Table 6.5. Endpoints compared between TCZ and placebo groups in the interim analysis.

Pre-challenge FEV_1 ($FEV_{1,baseline}$) and the minimum FEV_1 measured 3 to 7 hours post-allergen (LAR) from the screening and treatment challenges were used to calculate the LAR $\%fall_{max}$ as indicated in the Methods. For example, for patient P210300001, LAR $\%fall_{max}$ = (1-(2.57/3.07))-(1-(2.25/3.07)).

Analysis of safety endpoints

Adverse events (AEs) and serious adverse events (SAEs)

Nine AEs (Table 6.6) and one SAE (campylobacter enteritis between visits 1 and 2) were recorded amongst the 55 participants who enrolled in the trial but did not continue to the treatment phase. Fifteen AEs were reported for five of the 11 participants who completed the trial (Table 6.6). Most of these occurred during or after drug infusion (10 or 67%) and were mild. Only one AE (asymptomatic neutropenia) was determined to be related to the study drug, and one other AE (headache) was also probably related to the drug. These are common side-effects of tocilizumab that have previously been reported.[277,278] No SAE or malignancy was recorded in the 11 participants who completed the trial.

ID	Visit	AE / SAE	SAE	IP related?	Resolved?
Randomised to treatment					
Placebo					
S00030	3	Vasovagal event	No	No	Yes
S00030	9	Sore throat	No	No	Yes
S00030	9	Headache	No	No	Yes
S00028	10	Mild fever	No	No	Yes
Tocilizumab					
S00023	1	Wisdom tooth extraction	No	No	Yes
S00023	3	Headache	No	No	Yes
QPS0100	4	Migraine	No	Probably	Yes
S00023	4	Headache	No	No	Yes

S00023	5	Pre-syncopal event	No	No	Yes
S00023	6	Headache	No	No	Yes
QPS0100	7	Mild neutropenia	No	Definitely	Yes
QPS0100	7	Otitis externa (earache)	No	No	Yes
S00013	7	Headache	No	No	Yes
S00013	7	Knee injury	No	No	Yes
S00013	9	Medial tibial stress syndrome (shin splints)	No	No	Yes
Not randomised to treatment					
QPS0127	1	Campylobacter enteritis	Yes	No	Yes
QPS0103	2	Right eye inflammation	No	No	Yes
QPS0127	2	Muscle pain	No	No	Yes
S00002	2	Headache	No	No	Yes
S00019	2	Pre-syncopal event	No	No	Yes
S00027	2	Left knee staph infection	No	No	Yes
S00029	2	Suspected food poisoning	No	No	Yes
S00034	2	Mild fever	No	No	Yes
S00019	4	Headache	No	No	Yes
S00019	4	Suspected viral respiratory infection	No	No	Yes

Table 6.6. List of adverse events (AEs) and serious AEs (SAEs) reported in subjects enrolled in the study.

Laboratory evaluations – blood cell counts

In the tocilizumab group, neutrophil counts decreased significantly between the screening and post-treatment visits (Figure 6.2). For example, comparing visit 3 (first allergen challenge) against visit 7 (second allergen challenge), neutrophil counts decreased on average from 3.4 (SD = 1.22) to 2.5 (SD = 1.50), a statistically significant difference (P = 0.0313; Table 6.7). Similar results were observed for total white blood cell (WBC) counts. In the placebo group, these effects were not observed (Table 6.7): neutrophil (and WBC) did not change significantly within-subjects between pre- and post-treatment visits.

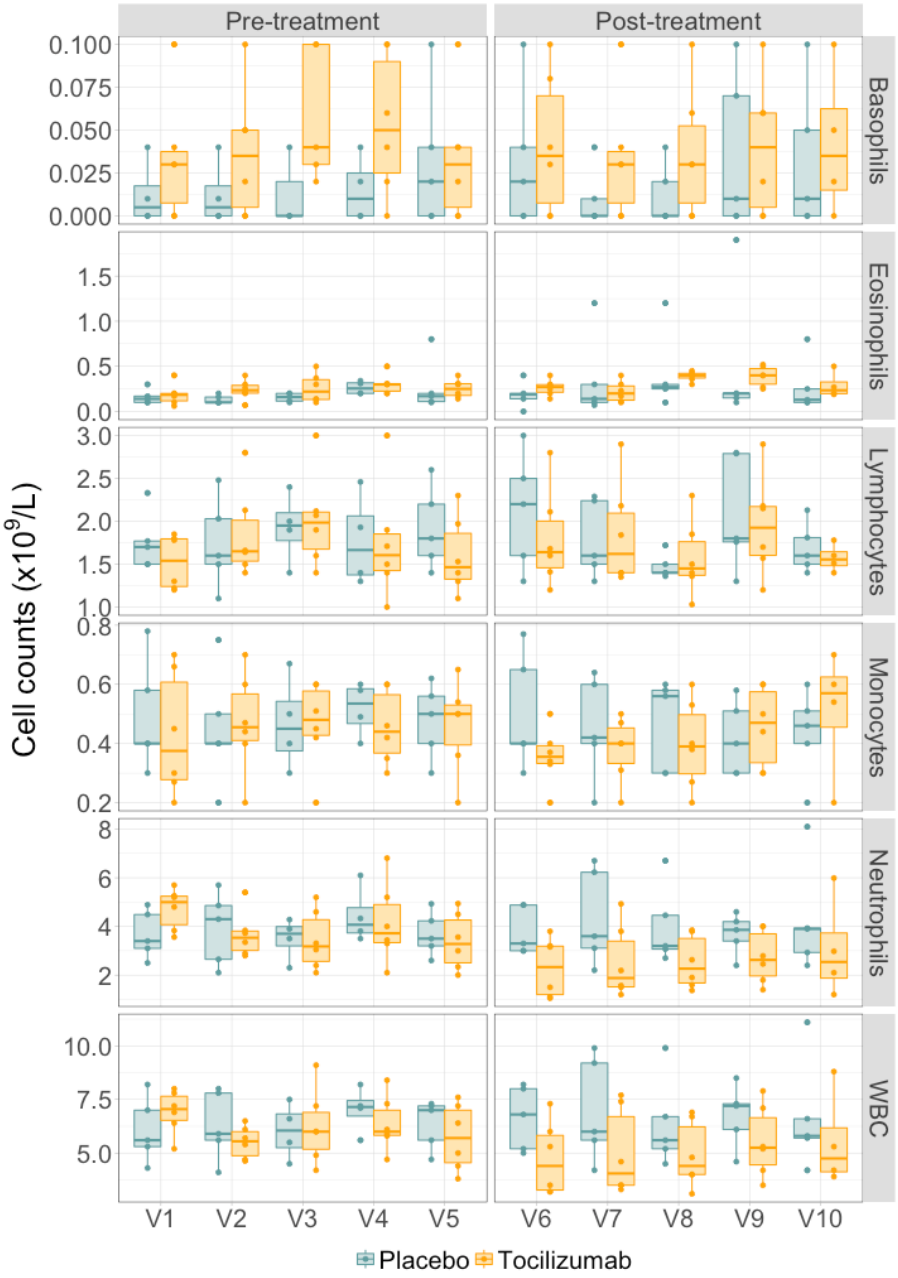


Figure 6.2. White blood cell counts measured before, and after infusion with placebo or tocilizumab.

Abbreviations: V, visit; WBC, white blood cells.

	V1 vs. V5	V2 vs. V6	V3 vs. V7	V4 vs. V8	V5 vs. V9
Tocilizumab					
Basophils	0, 0, 1	0, 0, 0.7893	0.1, 0, 0.1814	0.1, 0, 0.1003	0, 0, 0.3458
Eosinophils	0.2, 0.3, 0.0591	0.2, 0.3, 0.1814	0.3, 0.2, 0.1775	0.3, 0.4, 0.0585	0.3, 0.4, 0.0313
Lymphocytes	1.5, 1.6, 0.5992	1.9, 1.8, 0.1362	2, 1.8, 0.0938	1.8, 1.6, 0.0625	1.6, 2, 0.0313
Monocytes	0.4, 0.5, 0.5896	0.5, 0.4, 0.0568	0.5, 0.4, 0.0579	0.5, 0.4, 0.0591	0.5, 0.5, 1
Neutrophils	4.7, 3.4, 0.0355	3.7, 2.3, 0.0313	3.4, 2.5, 0.0313	4.1, 2.5, 0.0313	3.4, 2.7, 0.0313

WBC	6.9, 5.7, 0.0313	5.5, 4.8, 0.1563	6.2, 5, 0.0585	6.4, 4.9, 0.0313	5.7, 5.5, 0.5992
Placebo					
Basophils	0, 0, 0.1814	0, 0, 0.1814	0, 0, -	0, 0, -	0, 0, 1
Eosinophils	0.2, 0.3, 0.0975	0.1, 0.2, 0.4164	0.2, 0.4, 0.625	0.3, 0.5, 0.25	0.3, 0.5, 0.1814
Lymphocytes	1.8, 1.9, 0.1696	1.7, 2.1, 0.0625	1.9, 1.7, 0.0975	1.8, 1.5, 0.4227	1.9, 2.1, 0.2012
Monocytes	0.5, 0.5, 0.7893	0.4, 0.5, 0.1814	0.5, 0.5, 1	0.5, 0.5, 1	0.5, 0.4, 0.1003
Neutrophils	3.7, 3.7, 0.5827	3.9, 3.8, 1	3.5, 4.7, 0.25	4.4, 4.4, 0.875	3.7, 3.7, 1
WBC	6.1, 6.4, 0.4375	6.3, 6.6, 0.3125	6, 7.2, 0.375	7, 6.8, 0.875	6.4, 6.7, 0.1875

Table 6.7. Differences in white blood cell (WBC) counts before and after tocilizumab.

Results are expressed as mean at screening visit, mean at treatment visit, P-value from paired Wilcoxon test. Cell counts are in cell x 10^9/l. Abbreviations: V, visit.

There were no noteworthy treatment effects on other white blood cell counts measured (Figure 6.2 and Table 6.7).

In RA and Crohn's disease clinical trials, haemoglobin levels have been reported to increase with tocilizumab therapy.[279-281], [282,283] IL-6 promotes hepcidin production, which in turn restricts iron availability.[284,285] Thus, blockade of IL-6R was expected to decrease hepcidin levels and increase iron availability for the production of haemoglobin. In our study, haemoglobin levels were significantly (P = 0.0313; Figure 6.3; Table 6.8) lower on the day of infusion (visit 5) compared to the day patients were first assessed (visit 1). However, in visit 5 blood was drawn before infusion, so both these measurements were made before treatment and are not related to tocilizumab. In the post-treatment visits, haemoglobin levels fluctuated, but there were no significant differences compared to baseline visits.

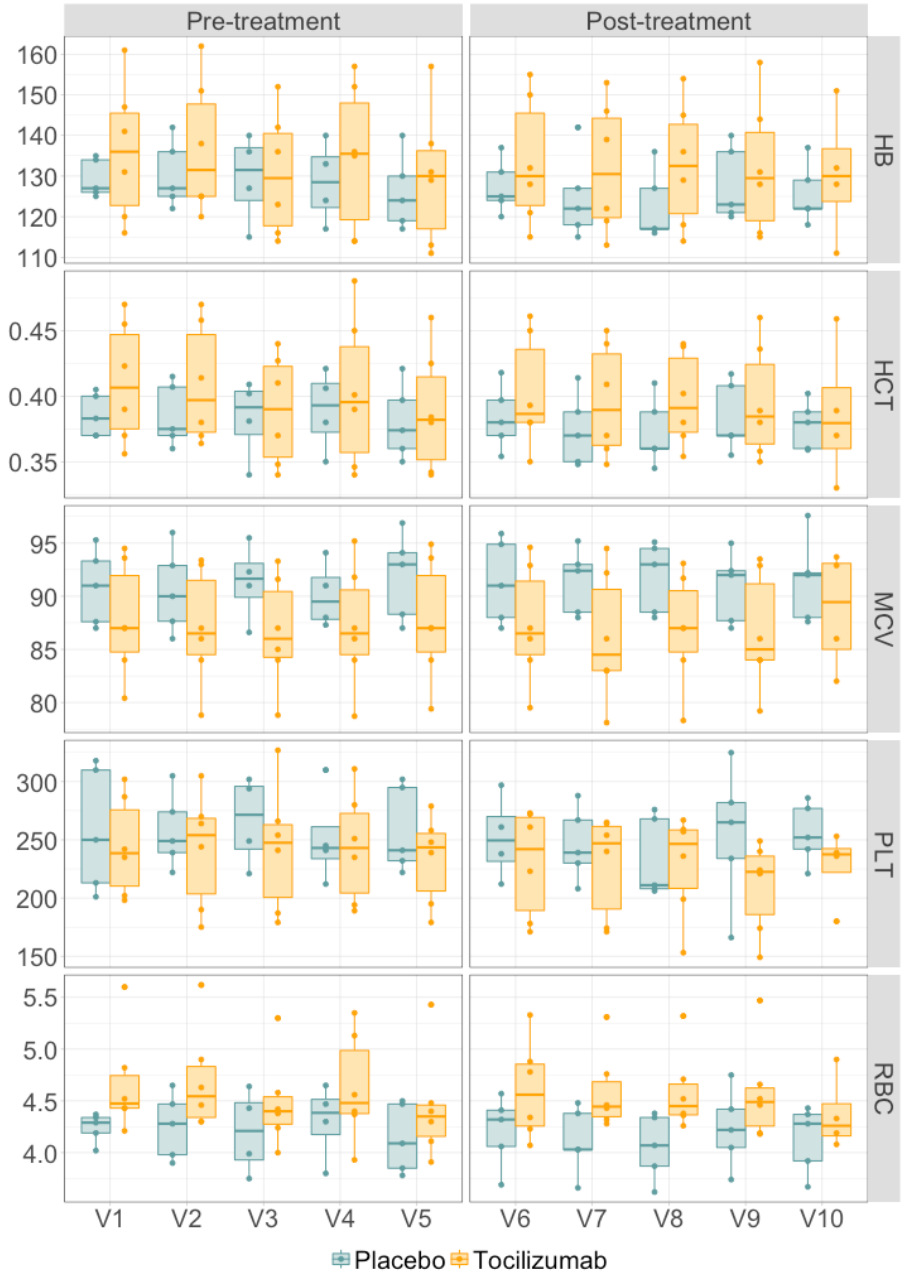


Figure 6.3. Haematology results before, and after infusion with placebo or tocilizumab.

Abbreviations: HB, haemoglobin (g/l); HCT, haematocrit (%); MCV, mean cell volume (fl); PLT, platelets (x 10^9/l); RBC, Red blood cells (x 10^{12}/l); V, visit.

	V1 vs. V5	V2 vs. V6	V3 vs. V7	V4 vs. V8	V5 vs. V9
Tocilizumab					
Haemoglobin, g/l	136, 129.8, 0.0313	136.8, 133.5, 0.0938	130.5, 132, 0.2021	134.7, 132.7, 0.3613	129.8, 132, 0.1362
Haematocrit, %	0.4, 0.4, 0.0313	0.4, 0.4, 0.2807	0.4, 0.4, 0.1056	0.4, 0.4, 1	0.4, 0.4, 0.1003
Mean cell volume, fl	87.8, 87.7, 1	87, 87.3, 0.4227	86.6, 86.1, 0.4004	87.1, 86.8, 0.5839	87.7, 86.6, 0.0591
Platelets, x 10^9/l	244.3, 233, 0.1563	241.3, 229.7, 0.0625	242.3, 228, 0.0591	243.3, 228.5, 0.4375	233, 209.5, 0.035
Red cell count, x 10^{12}/l	4.7, 4.4, 0.0313	4.7, 4.6, 0.2188	4.5, 4.6, 0.0313	4.6, 4.6, 0.3125	4.4, 4.6, 0.0313
Placebo					
Haemoglobin, g/l	129.4, 126, 0.3125	130.4, 127.4, 0.0568	129.5, 127.2, 0.625	128.5, 124.2, 0.1814	126, 128, 0.2012
Haematocrit, %	0.4, 0.4, 0.4375	0.4, 0.4, 0.5839	0.4, 0.4, 0.625	0.4, 0.4, 0.25	0.4, 0.4, 0.2785
Mean cell volume, fl	90.8, 91.9, 0.1003	90.5, 91.4, 0.1041	91.3, 92.3, 0.25	90.1, 91, 0.125	91.9, 90.8, 0.1003
Platelets, x 10^9/l	258.4, 258.4, 0.8125	266.8, 252, 0.125	266.5, 248.2, 0.125	252, 225.2, 0.0975	258.4, 254.4, 1
Red cell count, x 10^{12}/l	4.2, 4.1, 0.2785	4.3, 4.2, 0.4375	4.2, 4.1, 0.25	4.3, 4.2, 0.25	4.1, 4.2, 0.3125

Table 6.8. Differences in haematology results before and after tocilizumab.

Results are expressed as mean at screening visit, mean at treatment visit, P-value from paired Wilcoxon test. Abbreviations: V, visit.

Laboratory evaluations – blood biochemistry

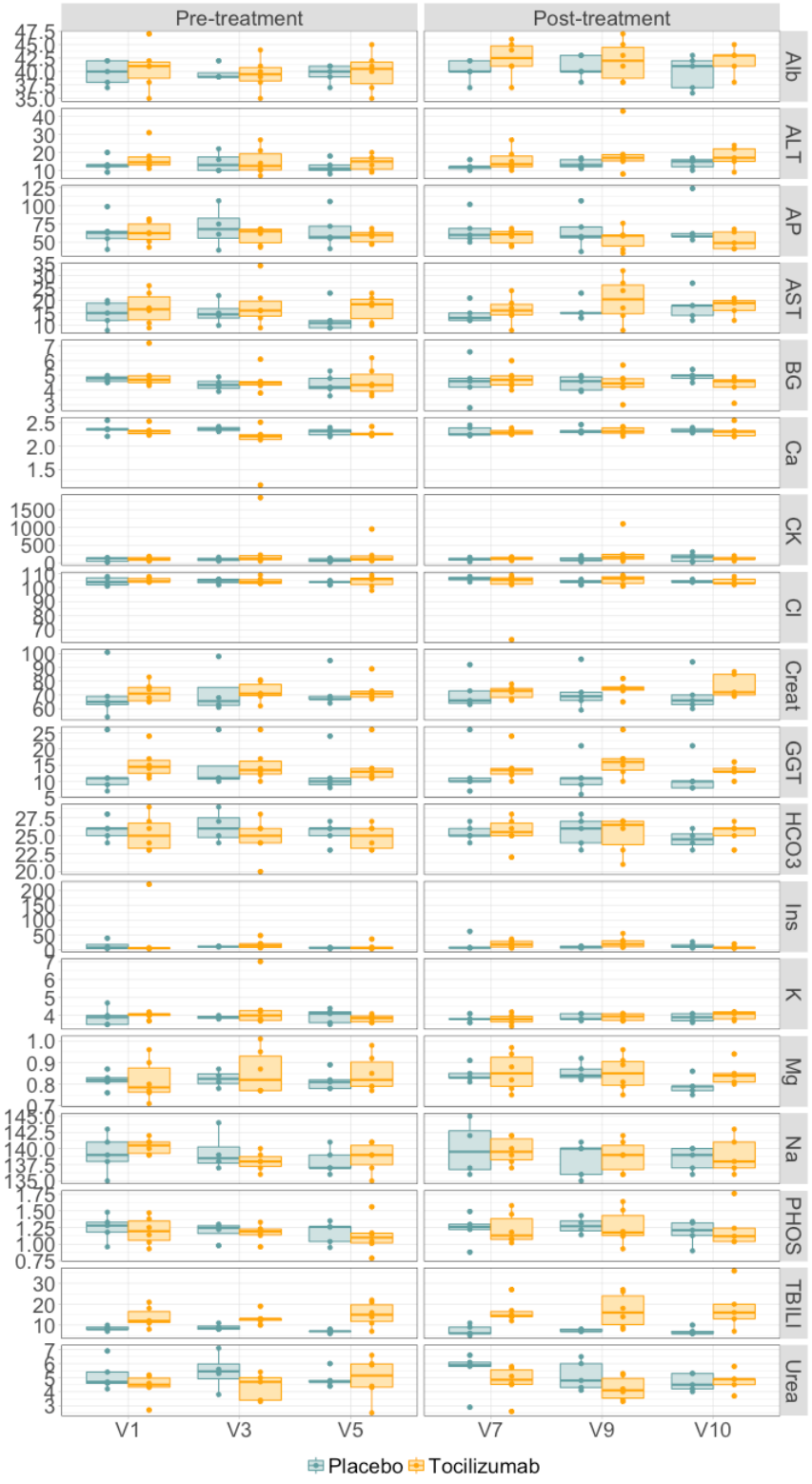


Figure 6.4. Blood biochemistry results before, and after infusion with placebo or tocilizumab.

Abbreviations: Alb, albumin (g/l); ALT, alanine transaminase (U/l); AP, alkaline phosphatase (U/l); AST, aspartate transaminase (U/l); BG, glucose (mmol/l); Ca, calcium (mmol/l); CK, creatine kinase

(U/l); Cl, Chloride (mmol/l); Creat, creatinine (µmol/l); GGT, gamma-glutamyl transpeptidase (U/l); HCO3, bicarbonate (mmol/l); Ins, insulin (pmol/l); K, potassium (mmol/l); Mg, magnesium (mmol/l); Na, sodium (mmol/l); PHOS, phosphate (mmol/l); TBILI, bilirubin total (µmol/l); Urea (mmol/l); V, visit.

In the tocilizumab group, albumin levels increased significantly after treatment (Figure 6.4). Specifically, levels measured before treatment were on average 39.5 (visit 3) and 40 g/l (visit 5), and increased to 42.3 (visit 7) and 42.0 g/l (visit 9) after treatment (Table 6.9). Bilirubin increased from visit 3 (13.3 µmol/l) to visit 7 (16.5 µmol/l). These observations are in line with reports from previous clinical trials of tocilizumab.[283],[286,287] Electrolytes were generally stable before and after treatment. However, at 7 hours post allergen-inhalation, sodium levels increased from pre- (138 mmol/l) to post-treatment (139.7 mmol/l) with tocilizumab (P = 0.0335). Phosphate levels also increased from visit 5 (1.1 mmol/l) to visit 9 (1.3 mmol/l).

	V1 vs. V5	V3 vs. V7	V5 vs. V9
Tocilizumab			
Alanine transaminase, U/l	17, 14.3, 0.8923	15, 15.8, 0.7874	14.3, 19.8, 0.0739
Albumin, g/l	40.7, 40, 0.1736	39.5, 42.3, 0.0335	40, 42, 0.0345
Alkaline phosphatase, U/l	63.3, 58, 0.1718	59, 57.7, 0.3951	58, 54.8, 0.3125
Aspartate transaminase, U/l	17, 17, 0.5982	18.2, 16.2, 0.2809	17, 20.3, 0.4004
Bicarbonate, mmol/l	25.3, 24.8, 0.3711	24.7, 25.5, 0.089	24.8, 25.2, 0.7103
Bilirubin, µmol/l	13.7, 15.2, 0.1362	13.3, 16.5, 0.034	15.2, 17, 0.4375
Calcium, mmol/l	2.3, 2.3, 0.035	2.1, 2.3, 0.2072	2.3, 2.3, 0.1056
Chloride, mmol/l	105.3, 104.5, 1	104.8, 98.7, 0.5992	104.5, 105.5, 1
Creatine Kinase, U/l	113.7, 255, 0.5992	409, 123.2, 1	262, 334.6, 1
Creatinine, µmol/l	71.8, 73.2, 0.4568	72.3, 72, 0.9163	73.2, 74.3, 0.5271
Gamma-glutamyl transpeptidase, U/l	15.5, 14.7, 0.3408	15.3, 14.5, 0.3711	14.7, 16.3, 0.0993
Glucose, mmol/l	5.1, 4.6, 0.0938	4.6, 4.8, 0.1975	4.6, 4.4, 0.2932
Insulin, mU/l	41, 11.1, 0.8438	18.7, 19.6, 1	11.1, 23.8, 0.4375
Magnesium, mmol/l	0.8, 0.8, 0.034	0.9, 0.9, 1	0.8, 0.9, 1
Phosphate, mmol/l	1.2, 1.1, 0.1563	1.2, 1.2, 0.5625	1.1, 1.3, 0.0313
Potassium, mmol/l	4, 3.8, 0.0313	4.5, 3.8, 0.0591	3.8, 3.9, 0.0975
Sodium, mmol/l	140.3, 138.7, 0.0568	138, 139.7, 0.0335	138.7, 138.8, 0.7728
Urea, mmol/l	4.4, 5, 0.2012	4.4, 5.1, 0.0579	5, 4.2, 0.1148
Placebo			
Alanine transaminase, U/l	13.4, 12, 0.089	14.5, 12.8, 0.625	12, 13.8, 0.5807
Albumin, g/l	39.8, 39.6, 0.7728	39.8, 40.2, 0.7893	39.6, 40.8, 0.0947
Alkaline phosphatase, U/l	64.4, 66.2, 0.5879	70.5, 70.2, 0.875	66.2, 65.8, 1
Aspartate transaminase, U/l	14.8, 12.8, 0.6845	15.2, 15.2, 1	12.8, 16.2, 0.4375
Bicarbonate, mmol/l	25.8, 25.4, 0.3458	26.2, 25.2, 0.3458	25.4, 25.6, 0.7728
Bilirubin, µmol/l	8.4, 7, 0.089	9, 8, 0.3458	7, 7.4, 0.3458
Calcium, mmol/l	2.4, 2.3, 0.0625	2.4, 2.3, 0.4227	2.3, 2.3, 0.3125
Chloride, mmol/l	104.4, 103.8, 0.5807	104.7, 105.7, 0.3711	103.8, 104.2, 0.3458
Creatine Kinase, U/l	97.4, 81, 1	102.5, 118, 1	81, 105, 0.0625

Creatinine, µmol/l	70.4, 72.4, 0.5879	72.5, 73.8, 0.8539	72.4, 72.4, 0.8923
Gamma-glutamyl transpeptidase, U/l	12.8, 12.4, 0.5862	14.5, 14.2, 1	12.4, 11.6, 0.4227
Glucose, mmol/l	4.8, 4.4, 0.1875	4.4, 4.1, 0.4227	4.4, 4.5, 0.4982
Insulin, mU/l	14.7, 6.6, 0.625	11, 6.8, 0.125	6.5, 9.3, 0.3125
Magnesium, mmol/l	0.8, 0.8, 1	0.8, 0.8, 0.125	0.8, 0.9, 0.0625
Phosphate, mmol/l	1.2, 1.2, 0.0625	1.2, 1.2, 0.7893	1.2, 1.3, 0.25
Potassium, mmol/l	3.9, 4, 0.8551	3.9, 3.7, 0.25	4, 3.9, 0.625
Sodium, mmol/l	139.2, 138, 0.129	139.7, 139.3, 0.7728	138, 138.4, 0.8501
Urea, mmol/l	5.2, 4.9, 0.5839	5.4, 6.1, 0.2693	4.9, 5.1, 0.625

Table 6.9. Differences in haematology results before and after tocilizumab.

Results are expressed as mean at screening visit, mean at treatment visit, P-value from paired Wilcoxon test. Abbreviations: V, visit.

Analysis of primary efficacy endpoints

Bronchoconstriction during the LAR induced by allergen challenge

We analysed two primary efficacy endpoints, both of which measure the magnitude of bronchoconstriction recorded between 3 and 7 hours after allergen challenge (i.e. during the LAR): LAR $\%fall_{max}$ and $AUC_{3\text{-}7h}$. Details on how these were calculated for each individual are provided in detail in the Methods section. Briefly, LAR $\%fall_{max}$ was calculated as the difference between the largest drop in FEV_1 measured 3 – 7h in post- and pre-treatment allergen challenges.

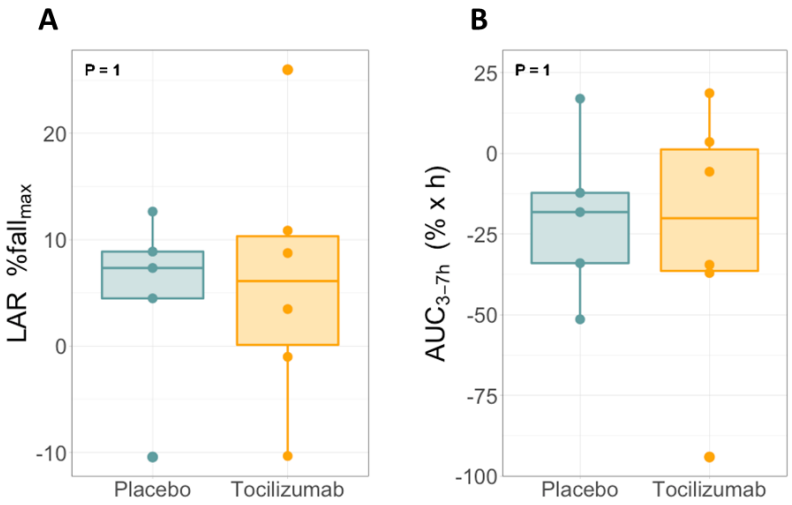


Figure 6.5. Comparison of LAR $\%fall_{max}$ (A) and $AUC_{3\text{-}7h}$ (B) between patients treated with placebo or tocilizumab.

LAR $\%fall_{max}$ was not significantly different between the tocilizumab (n = 6, mean= 6.3%, SD = 12.3%) and placebo (n = 6, mean = 4.5%, SD = 8.9%) groups (P = 1; Figure 6.5 A). Similarly,

there were no significant differences between groups for the AUC3-7h (Figure 6.5 B). Thus, tocilizumab did not significantly influence the primary endpoints measured in this study.

Overall, FEV_1 fluctuations throughout the pre- a post-treatment allergen inhalation challenges tests were comparable between the two treatment groups (**Figure 6.6**).

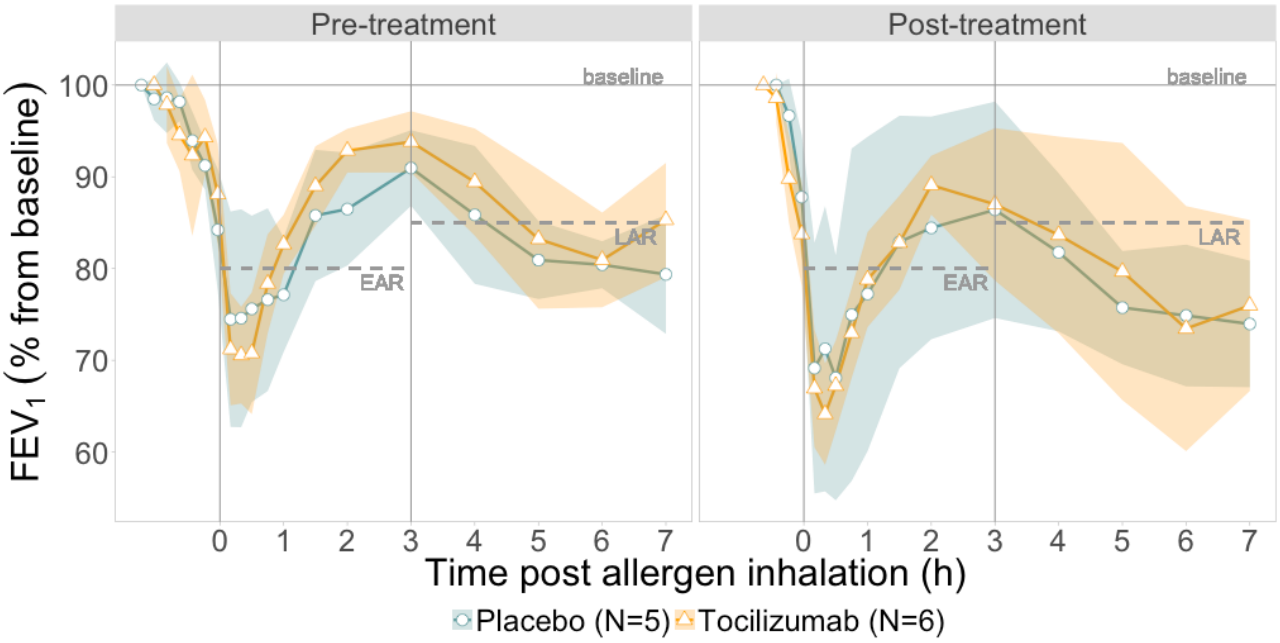


Figure 6.6. Spirometry measurements in the pre- and post-treatment allergen challenges.

Solid lines represent average FEV_1. Shading represents 95% CI around the mean. Abbreviations: EAR, early asthmatic response; LAR, late asthmatic response.

Analysis of secondary efficacy endpoints

Bronchoconstriction induced during the early asthmatic response (EAR)

To test if tocilizumab improved the immediate bronchoconstriction triggered by allergen challenge, for each individual we measured the EAR $\%fall_{max}$, that is, the difference between the post- and pre-treatment allergen challenges in the largest FEV_1 % drop measured 0 - 3h after allergen challenge (see Methods for details). As for EAR $\%fall_{max}$, we found that EAR $\%fall_{max}$ was not significantly different between the placebo and tocilizumab groups (P = 0.5368; Figure 6.7 A). Similarly, no differences were observed between groups when bronchoconstriction during the EAR was measured using $AUC_{0\text{-}3h}$ (P = 0.3290; Figure 6.7 B).

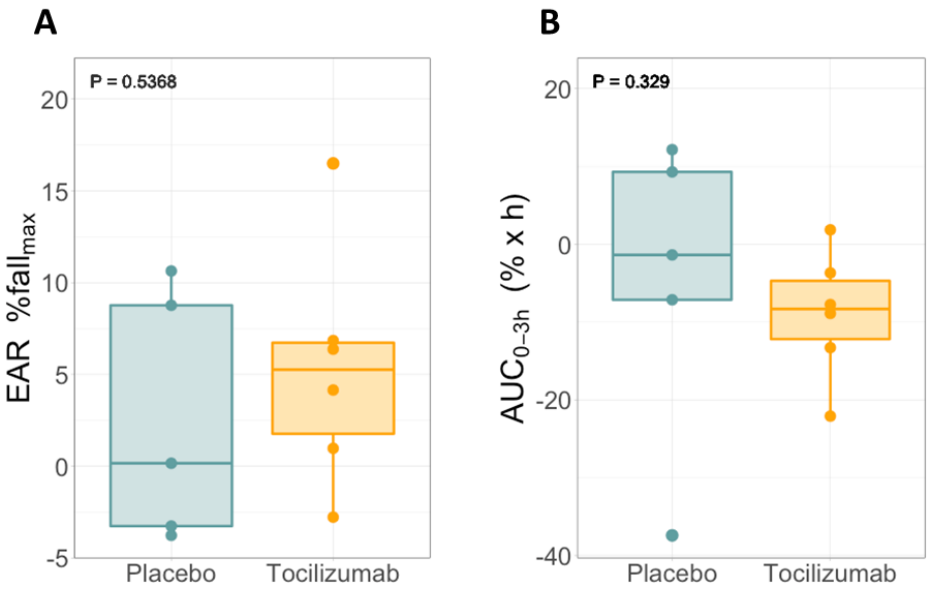


Figure 6.7. EAR %fall$_{max}$ (A) and AUC$_{0\text{-}3h}$ (B) in patients treated with placebo or tocilizumab.

Airway hyperresponsiveness (AHR) to methacholine, before and after allergen challenge

The methacholine PC_{20} measured 24 hours prior to allergen challenge provides a measure of AHR that is independent of an inflammatory response triggered by allergen exposure. To test if tocilizumab had any effect on AHR, for each participant we calculated ΔPC_{20} as the difference in PC_{20} between the post- and pre-treatment allergen challenges. We found no significant differences in ΔPC_{20} between the tocilizumab and placebo groups (P = 0.3290, Figure 6.8).

We then repeated the same analysis but considered the methacholine PC_{20} observed 24 hours after the allergen challenge, which now also captures the persistence of airway inflammation triggered by the allergen challenge in the previous day. Again, we observed no difference in the delta PC_{20} after allergen challenge between tocilizumab and placebo groups (P = 0.6857; Figure 6.8).

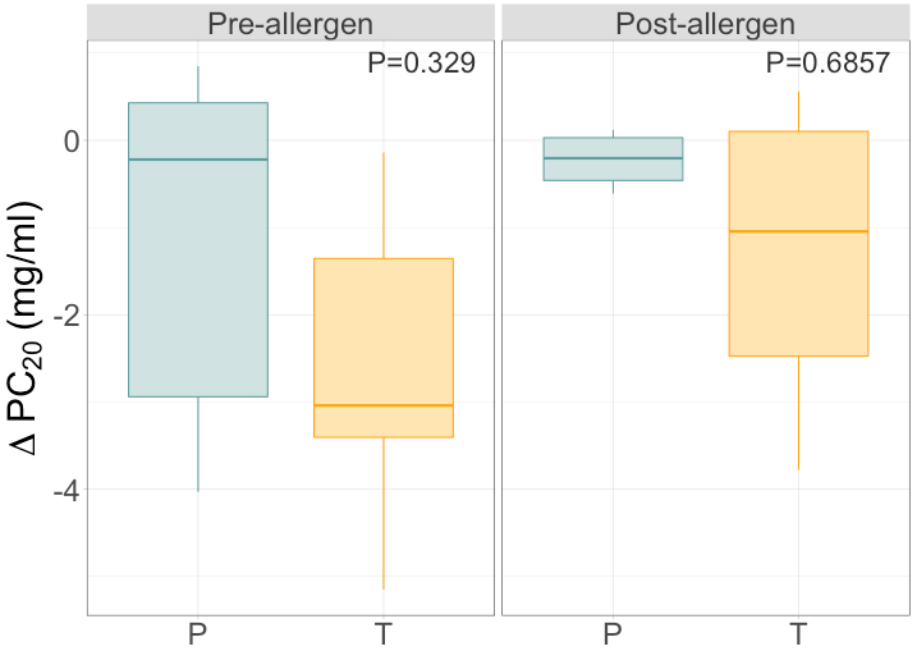


Figure 6.8. ΔPC_{20} measured pre- and post-allergen challenge.

ΔPC_{20} was calculated for each participant as the difference between the PC_{20} measured after treatment and before treatment (e.g. $\Delta PC_{20\ Pre\text{-}allergen} = PC_{20\ Pre\text{-}allergen,treatment} - PC_{20\ Pre\text{-}allergen,screening}$).

Immune cell counts in induced sputum samples

We also compared immune cell counts from sputum samples obtained before and after treatment. When we compared visit 2 against visit 6, there were no significant differences in Δ cell counts between tocilizumab and placebo groups for any of the four immune cell types analysed (Table 6.10; Figure 6.9). Similar results were obtained when comparing visit 3 against visit 7, and visit 4 against visit 8.

	V6-V2			V7-V3			V8-V4		
	TCZ	Placebo	*P*-value	TCZ	Placebo	*P*-value	TCZ	Placebo	*P*-value
Macrophages	-0.78	0.75	0.40	0.20	-1.26	0.80	-0.38	-1.69	0.70
Neutrophils	0.06	-1.61	1.00	-5.35	0.93	0.20	-1.89	-5.09	0.40
Eosinophils	-0.02	2.17	0.15	-0.42	0.22	0.40	-0.24	-0.71	0.70
Lymphocytes	-0.09	-0.08	0.86	-0.07	-0.02	0.40	-0.13	-0.28	1

Table 6.10. Comparison of Δ immune cell counts in sputum between tocilizumab and placebo groups.

The difference between visits (i.e. Δ) was calculated using number of cells per g of sputum. Abbreviations: TCZ, tocilizumab.

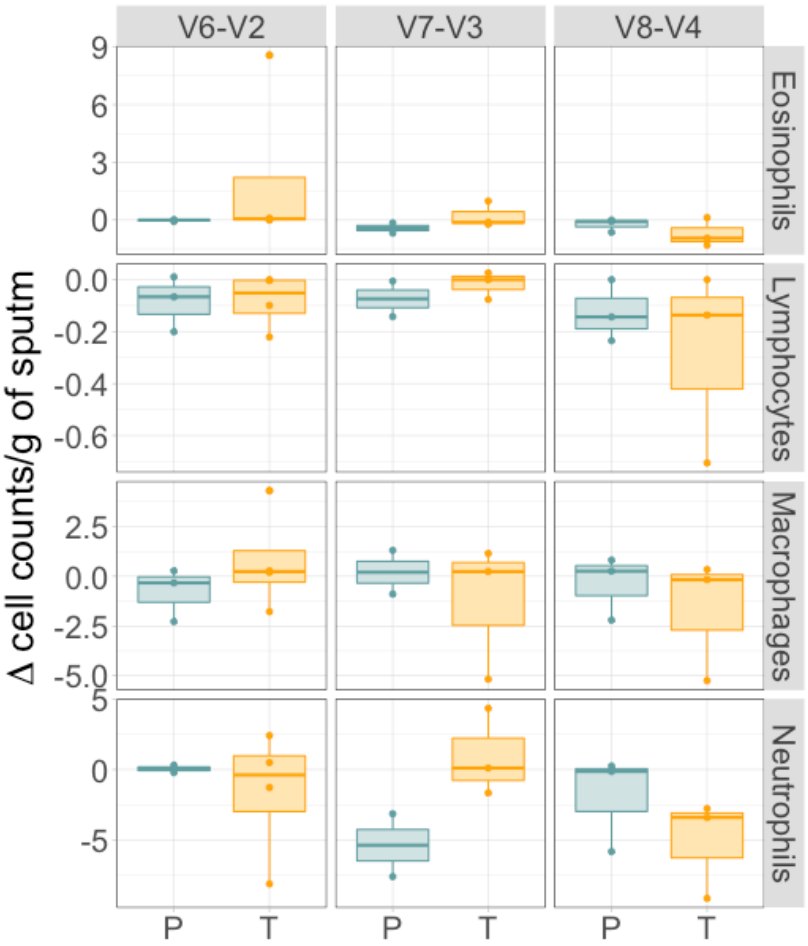


Figure 6.9. Comparison of Δ immune cell counts in sputum between tocilizumab and placebo groups.

Δ cell counts were calculated for each participant as the difference between the cell count measured after treatment and the cell count measured before treatment in the matching visit (e.g. $\Delta_{V6\ V2,eosinophils}$ = Eosinophil counts measured at visit 6 - Eosinophil counts measured at visit 2).

Serum and sputum inflammatory mediators

When comparing inflammatory mediators before and after treatment, large increases were observed in the tocilizumab group for serum IL-6 levels (Figure 6.10 and Figure 6.12). For example, in the pre-treatment phase, serum IL-6 levels were 0.2 and 0 pg/ml at 7 and 24 hours post allergen inhalation, respectively. After treatment, these figures changed to 12.2 and 13.4 pg/ml, respectively.

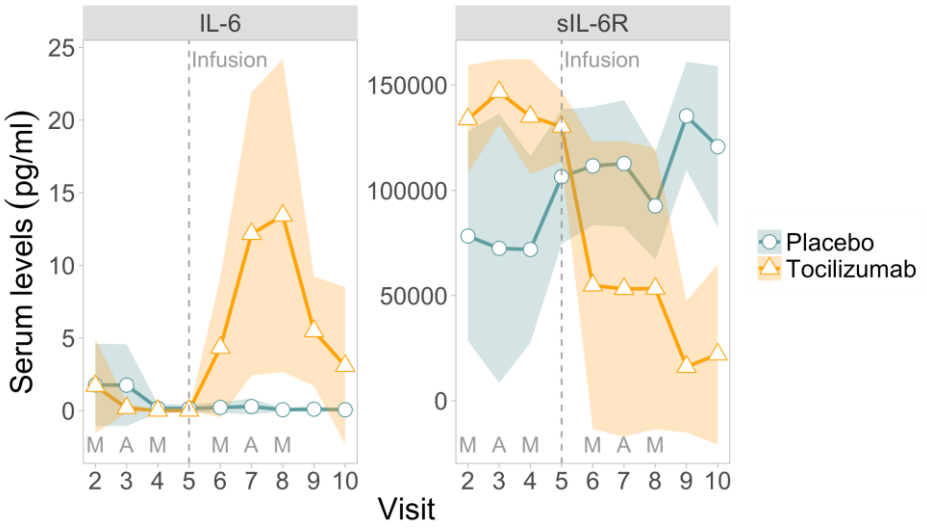


Figure 6.10. Serum IL-6 and sIL-6R levels in allergen inhalation challenge tests.

Solid lines represent average protein levels. Shading represents 95% CI around the mean. Abbreviations: A, allergen challenge; M, methacholine challenge.

We also observed decreases in C-reactive protein (CRP) serum levels, which were not seen with placebo. (Figure 6.12) However, none of these differences was statistically significant (Table 6.11). We did, however, observe a significant decrease in serum sIL-6R levels from visit 5 to visit 9 (130.2 vs. 16.2, $P = 0.0313$). Similarly, sIL-6R levels decreased from pre- to post-treatment in all other visits, but these differences were not statistically significant (Table 6.11). Conversely, sIL-6R levels measured in sputum increased from pre- to post-treatment (Figure 6.11 and Figure 6.13). For example, sputum sIL-6R levels measured 7 hours after allergen inhalation were 357.3 pg/ml before treatment, and increased to 1,005.5 pg/ml after treatment. However, these differences were also not significant (Table 6.12) No other relevant differences were observed in sputum.

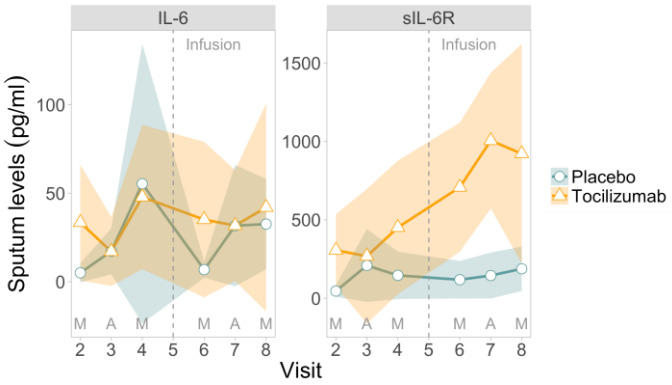


Figure 6.11. Sputum IL-6 and sIL-6R levels in allergen inhalation challenge tests.

Solid lines represent average protein levels. Shading represents 95% CI around the mean. Abbreviations: A, allergen challenge; M, methacholine challenge.

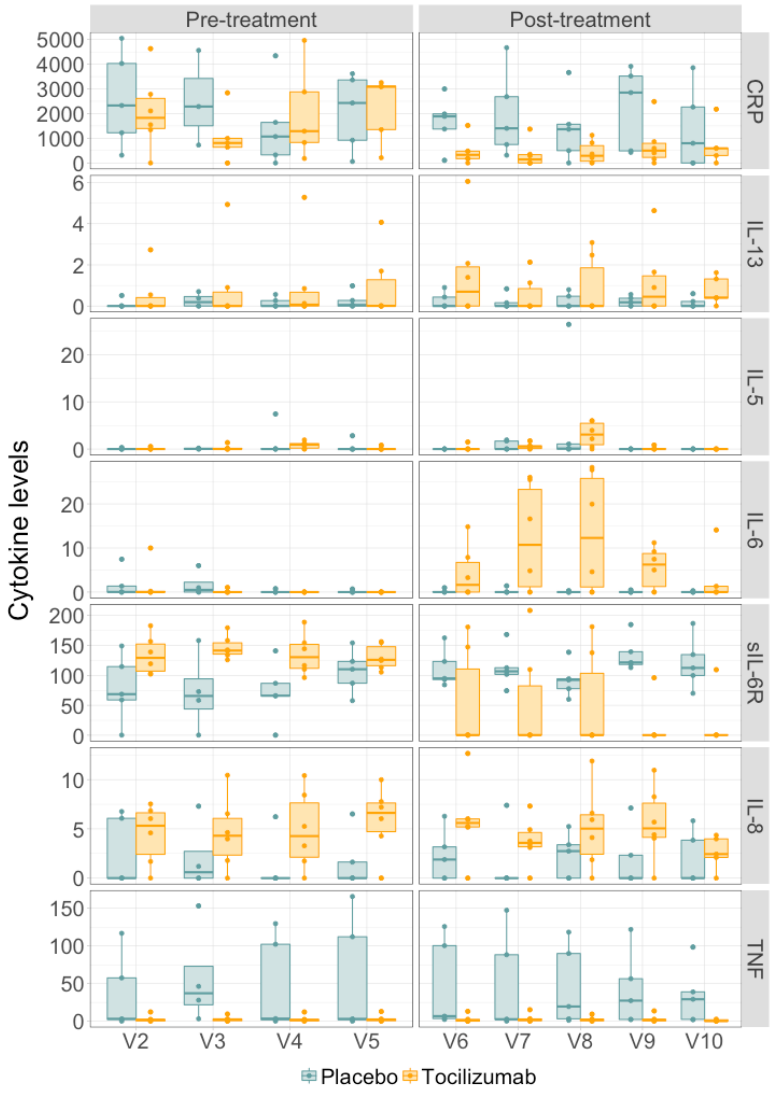


Figure 6.12. Serum cytokine levels before and after infusion with placebo or tocilizumab.

CRP and sIL-6R levels are presented in ng/ml. All other cytokines are presented in pg/ml. CRP, C-reactive protein; IL, interleukin; sIL-6R, IL-6 soluble receptor; TNF, tumour necrosis factor

	V2 vs. V6	V3 vs. V7	V4 vs. V8	V5 vs. V9
Tocilizumab				
CRP	2069.3, 471.7, 0.0625	1057.5, 128.8, 0.1003	2029.5, 437.4, 0.0625	2205.4, 822.3, 0.0625
IL-13	0.5, 1.6, 0.1814	1, 0.5, 0.7893	1, 0.9, 0.8551	1, 1.2, 0.8551
IL-5	0.1, 0.2, 1	0.3, 0.6, 0.4185	0.8, 3.1, 0.1056	0.1, 0.2, 0.3711
IL-6	1.7, 4.3, 0.1814	0.2, 12.2, 0.1056	0, 13.4, 0.1003	0, 5.5, 0.1003
sIL-6R	133.8, 54.8, 0.0938	146.7, 53.2, 0.0938	135, 53.3, 0.1563	130.2, 16.2, 0.0313
IL-8	4.4, 5.8, 0.4185	4.6, 3.7, 0.7874	4.9, 5.1, 1	5.9, 5.6, 1
TNF	3, 2.9, 0.8551	2.8, 3.8, 0.7874	3, 2.6, 0.5896	3.3, 3.3, 1
Placebo				
CRP	2588.8, 1672.5, 0.1875	2524.5, 1612.6, 1	1477.1, 1420.2, 1	2078.7, 2241.1, 1
IL-13	0.1, 0.3, 0.3711	0.3, 0.2, 1	0.2, 0.3, 0.3711	0.3, 0.2, 1
IL-5	0.1, 0, 1	0.1, 0.9, 0.4227	1.5, 5.5, 0.1814	0.6, 0, 1

IL-6	1.8, 0.2, 0.4227	1.7, 0.4, 1	0.2, 0.1, 1	0.1, 0.1, 1
sIL-6R	78.3, 111.6, 0.4375	72.4, 122.3, 0.125	71.9, 92.6, 0.3125	106.4, 135.3, 0.125
IL-8	2.6, 2.3, 1	2.1, 1.8, 1	1.2, 2.3, 0.4227	1.6, 1.9, 0.3711
TNF	35.9, 47.7, 0.4375	57.6, 59.6, 0.875	47.4, 46.4, 1	56.6, 42, 1

Table 6.11. Inflammatory mediators measured in serum.

Results are expressed as mean at screening visit, mean at treatment visit, P-value from paired Wilcoxon test. CRP and sIL-6R levels are presented in ng/ml. All other cytokines are presented in pg/ml. Abbreviations: CRP, C-reactive protein; IL, interleukin; sIL-6R, IL-6 soluble receptor; TNF, tumour necrosis factor; V, visit.

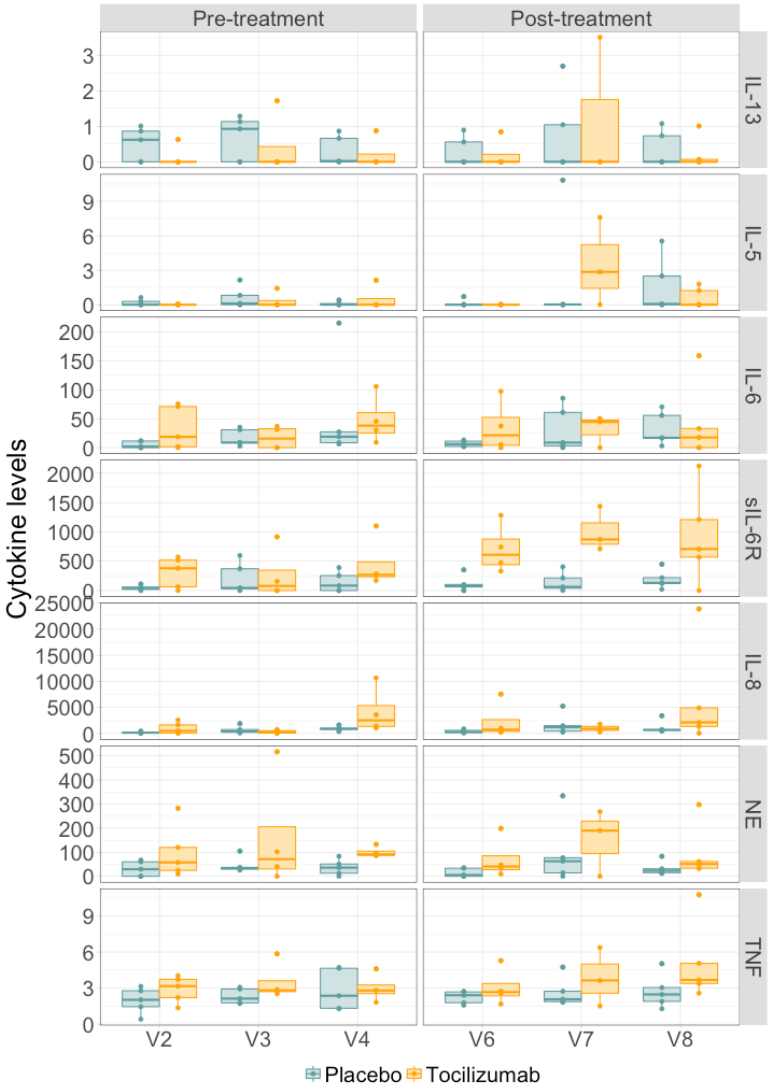


Figure 6.13. Sputum cytokine levels before and after infusion with placebo or tocilizumab.

NE levels are presented in ng/ml. All other cytokines are presented in pg/ml. IL, interleukin; sIL-6R, IL-6 soluble receptor; NE, neutrophil elastase; TNF, tumour necrosis factor.

	V2 vs. V6	V3 vs. V7	V4 vs. V8
Tocilizumab			
IL-13	0.2, 0.2, 1	0.6, 1.2, 1	0.2, 0.3, 0.3711
IL-5	0, 0, 1	0.5, 3.5, 0.3711	0.5, 0.8, 1
IL-6	41.8, 35.1, 0.875	22.7, 31.7, 0.3711	47.9, 52.5, 0.875
sIL-6R	382.9, 708.2, 0.625	357.3, 1005.5, 0.5	451.7, 975.7, 0.25
IL-8	1206, 2321.1, 0.875	419.1, 1002.3, 0.25	4192.1, 7558.5, 0.625
NE	117.6, 72.4, 0.125	206.4, 152.7, 1	100.3, 103.7, 0.875
TNF	2.8, 3.1, 0.875	3.8, 3.8, 1	3, 5.5, 0.25
Placebo			
IL-13	0.5, 0.3, 0.1814	0.7, 0.7, 1	0.3, 0.4, 0.4227
IL-5	0.2, 0.1, 1	0.6, 2.2, 1	0.1, 1.6, 0.5839
IL-6	5, 6.8, 0.4375	17.2, 31.7, 0.625	55.3, 32.6, 1
sIL-6R	45.8, 118.2, 0.1003	209, 144.8, 0.625	146, 187.9, 0.4375
IL-8	181.5, 414.7, 0.125	677.7, 1737.2, 0.4375	951.6, 1191, 0.8125
NE	31.4, 14.8, 0.1814	46.2, 97.7, 0.8125	36.7, 32.9, 0.4375
TNF	2, 2.2, 0.8125	2.3, 2.6, 0.625	2.9, 2.7, 1

Table 6.12. Inflammatory mediators measured in sputum.

Results are expressed as mean at screening visit, mean at treatment visit, P-value from paired Wilcoxon test. NE levels are presented in ng/ml. All other cytokines are presented in pg/ml. Abbreviations: IL, interleukin; sIL-6R, IL-6 soluble receptor; NE, neutrophil elastase; TNF, tumour necrosis factor; V, visit.

Sputum subtype after allergen challenge

Of the 11 participants who completed the trial, 8 provided a sputum sample 7 hours after allergen challenge in the screening phase. Sputum subtypes were eosinophilic (n = 7) or mixed granulocytic (n = 1). At 24 hours, 9 participants provided a sputum sample: paucigranulocytic, n = 1; eosinophilic, n = 5; neutrophilic, n = 1; and mixed granulocytic, n = 2. Sputum subtypes observed after treatment are listed in Table 6.13.

	Visit 2	Visit 3	Visit 4	Visit 6	Visit 7	Visit 8
P210300001	Pauci	-	Neutro	Pauci	Neutro	Pauci
P210300002	**Pauci**	**Eosino**	**Eosino**	**Pauci**	**Pauci**	**Pauci**
P210300003	Pauci	Eosino	Eosino	Pauci	Eosino	Eosino
P210300004	Pauci	Eosino	Eosino	Eosino	Pauci	Eosino
P210300005	**-**	**Eosino**	**MG**	**-**	**-**	**Eosino**
P210300006	**Pauci**	**Eosino**	**MG**	**MG**	**MG**	**MG**
QPS0100	**Eosino**	**Eosino**	**-**	**Eosino**	**Eosino**	**Eosino**
S00013	**Neutro**	**-**	**Eosino**	**Pauci**	**-**	**Eosino**
S00023	**-**	**-**	**-**	**-**	**-**	**-**
S00028	Eosino	MG	Eosino	Eosino	Eosino	Pauci
S00030	Pauci	Eosino	Pauci	Pauci	Pauci	Pauci

Table 6.13. List of sputum inflammatory subtypes in each study visit where sputum was induced.

Subjects treated with tocilizumab are represented in bold font. Abbreviations: Eosino, eosinophilic; Neutro, neutrophilic; MG, mixed granulocytic; Pauci, paucigranulocytic.

A single individual did not develop a LAR after treatment

Of note, one participant in the trial did not develop a LAR after treatment (LAR $\%fall_{max}$ < 15%). The subject was the only participant who (1) had mixed granulocytic sputum after the screening allergen challenge (when considering the sputum sample collected at 24 hours post challenge); (2) had the rs4129267:TT genotype; and (3) was in the tocilizumab group. No other participant met all three criteria. Furthermore, before treatment, the patient had the highest levels of sputum sIL-6R, and the highest, or second highest, levels of IL-6 in sputum collected at 7 and 24 hours post allergen inhalation (Figure 6.14). Specifically, sputum sIL-6R was 2- and 3.9-fold higher than average at 7 and 24 hours post-allergen, respectively. Sputum IL-6 was 2.2- and 3.9-fold higher than average at 7 and 24 hours post-allergen, respectively.

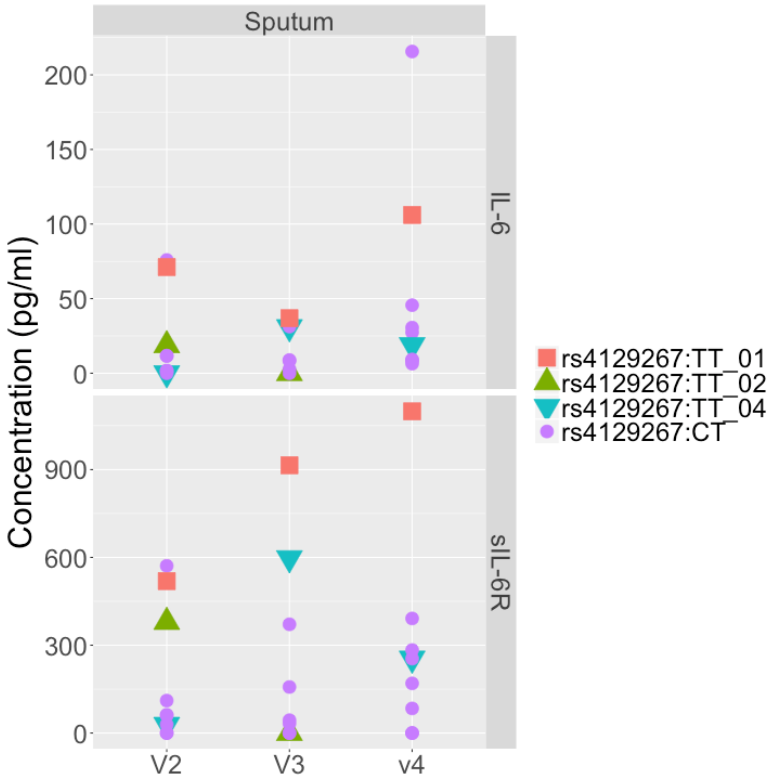


Figure 6.14. Sputum levels of IL-6 and sIL-6R measured in the pre-treatment phase, before (V2) and after (V3 and V4) allergen inhalation challenge.

The square represents cytokine levels from the only patient who did not have a LAR after treatment. Triangles represent cytokine levels from other patients with the rs4129267:TT genotype. Circles represent cytokine levels from subjects with the rs4129267:CT genotype. One patient with the rs4129267:TT genotype (rs4129267:TT_03) did not produce sputum in any of the study visits, and so has no cytokine measurements.

Discussion

An extensive body of evidence has implicated IL-6 signalling in asthma pathophysiology. Tocilizumab is a monoclonal antibody that blocks IL-6 signalling by binding to both mIL-6R and sIL-6R. In the current study, we evaluated the safety and efficacy of a single dose of 8 mg/kg of tocilizumab in allergen-induced asthmatic responses.

Safety assessment showed that the drug was generally well tolerated by asthma patients. A single drug-related adverse event was recorded, asymptomatic neutropenia. In addition, white blood cell counts were lower in tocilizumab-treated patients than in placebo-controls, mostly due to a significantly lower neutrophil count. Previous studies have reported significant decreases in neutrophil counts with tocilizumab treatment.[277,278] Thus, these were expected drug effects. Overall, our results show that tocilizumab is safe to treat patients with asthma. This is important because tocilizumab is now widely used to treat patients with rheumatoid arthritis – 1-7% of whom have been estimated to also suffer from asthma.[11,288,289]

In line with previous reports,[274,279,286] we also observed significant increases in serum IL-6 and sIL-6R levels after tocilizumab infusion. The high concentrations of IL-6 could be explained by an increase in free IL-6, i.e. IL-6 that is not bound to mIL-6R or sIL-6R. There is contradicting evidence on the possible effect of tocilizumab in dissociating IL-6 from IL-6/IL-6R complexes.[258,290] Nevertheless, continued production of IL-6 would be expected to drive increases in free IL-6, given that the remaining IL-6R would be blocked by tocilizumab. Another possible explanation for the increase in IL-6 levels is that tocilizumab blocks the IL-6R mediated clearance of IL-6. This hypothesis is supported by observations in models of experimental asthma.[290] Specifically, Uchiyama et al. showed that a single injection of tocilizumab in monkeys with collagen-induced arthritis did not induce IL-6 production synthesis (measured through IL-6 messenger RNA levels), but an anti-IL-6R antibody inhibited IL-6 clearance in IL-6 deficient mice continuously infused with human IL-6.[290]

The increase in serum levels of sIL-6R after tocilizumab treatment may be an artefact caused by tocilizumab binding to the ELISA assay that was used to measure sIL-6R levels. To our knowledge, this has not been tested. Another possible explanation, suggested by Nishimoto et al.,[291] is that tocilizumab increases the half-life of sIL-6R when sIL-6R/tocilizumab complexes are formed. This hypothesis is in line with previous observations that the half-life of antigens was increased in antibody/antigen complexes.[292]

Serum IL-6 levels measured in the screening phase were very low (Table 6.4), considering the concentrations observed in sputum and the correlation that we previously observed between serum and sputum levels of IL-6 (Figure 2.3). Two possible explanations for this include the timing of sample collection or biases in cytokine measurements. In the current study, baseline sputum measurements were performed in samples that were collected after the first methacholine challenge. Conversely, in chapter 2, serum and sputum collection were not preceded by any challenge tests. Thus, the timing of sample collection could have affected cytokine levels. This hypothesis is, however, unlikely. Firstly, methacholine challenge has been shown to have no effect on sputum inflammatory cell counts and biochemical biomarkers.[293] Secondly, blood samples were collected immediately after the methacholine challenge; thus, it is unlikely that cytokine levels measured in serum have been affected in that time frame. A more plausible explanation is that IL-6 concentrations were not accurately measured. Specifically, high concentrations of sIL-6R (> 20 ng/ml) may interfere with the detection of IL-6 in the ELISA kits used in this study. Thus, given the elevated concentration of sIL-6R observed in serum samples ($\bar{\bar{x}}_{\text{Placebo}}$ = 91.6 ng / ml; $\bar{\bar{x}}_{\text{Tocilizumab}}$ = 147.8 ng / ml), it is likely that IL-6 levels measured in serum are underestimated.

The primary efficacy outcome assessed in this study was the LAR $\%\text{fall}_{\text{max}}$, taken as the difference between post- and pre-treatment maximum FEV_1 % drop measured 3 – 7h after allergen inhalation. Overall, there were no differences in LAR $\%\text{fall}_{\text{max}}$ between the tocilizumab and placebo groups. After treatment, all but one participant experienced a LAR, defined by an FEV_1 fall > 15% from baseline, between 3 and 7 hours post allergen-inhalation. The exception was a patient with mixed granulocytic sputum after allergen challenge, homozygous for rs4129267:T and treated with tocilizumab. Interestingly, the participant also had higher airway levels of IL-6 and sIL-6R after allergen challenge. This is consistent with previous evidence from mouse models of experimental asthma that showed that IL-6R blockade is able to attenuate airway inflammation that is associated with elevated levels of IL-6 and sIL-6R. Our findings support the hypothesis that the pathological contribution of IL-6 to asthma is mainly due to the trans-signalling pathway. Curiously, a recent

evaluation of tocilizumab affinity suggests that the drug might be more efficient at blocking the classical signalling pathway. Specifically, tocilizumab affinity was found to be three times greater to mIL-6R than to sIL-6R.[294] This observation raises the possibility that at the dose used (8-mg/kg), tocilizumab might not have efficiently blocked sIL-6R. Previously, fortnightly intravenous infusions of 8 mg/kg of tocilizumab were shown to increase serum levels of sIL-6R and, while free tocilizumab (i.e. not bound to IL-6R) remained detectable in serum (i.e. above 1 μg/ml), over 95% of sIL-6R was found in the form of sIL-6R/tocilizumab immune complexes.[291] Although the degree of mIL-6R blockade was not assessed in that study, the recent observations on tocilizumab affinity suggest that most, if not all, mIL-6R will also be blocked with an 8 mg/kg monthly dose of tocilizumab, as long as the drug remains above 1 μg/ml in serum. In our study, free tocilizumab in serum was not measured. However, pharmacodynamics was monitored through the levels of serum IL-6, sIL-6R, and C-reactive protein (CRP). In line with previous reports,[256,291,295] we observed significant increases in IL-6 and sIL-6R, and decreases in CRP, after tocilizumab infusion. Therefore, our observations suggest that mIL-6R and sIL-6R were efficiently targeted at systemic level. However, it is possible that the amount of tocilizumab that reached the airways was lower than that observed at systemic level. This hypothesis is corroborated by the fact that there was no increase in airway levels of IL-6 after tocilizumab infusion. Thus, if tocilizumab levels were lower in the airways, we would expect mIL-6R to be preferentially targeted, which could explain why the drug only improved allergen-induced bronchoconstriction in the subject with higher airway levels of sIL-6R (and consequently lower mIL-6R levels). An alternative way to test the effect of tocilizumab in asthma patients would be to administrate the drug directly to the airways, for example through aerosol inhalation. However, the only two routes currently approved for tocilizumab administration are intravenous (used in this study) or subcutaneous infusions. Further studies are required to assess the feasibility of targeting the IL-6 pathway directly in the airways.

A relevant observation in the results above is that the subject who responded to tocilizumab had two copies of the rs4129267:T allele, which is estimated to increase serum sIL-6R levels by about 1.4-fold per copy,[27] and asthma risk by 1·09-fold.[1] Based on the effects of this variant, we previously hypothesised that tocilizumab could either worsen or improve asthma symptoms, depending on which pathway (classical or trans-signalling) was underlying the association between rs4129267 and asthma risk. Specifically, if rs4129267:T increases asthma risk because it decreases classical signalling, then we would expect asthma symptoms to worsen with tocilizumab, which blocks signalling through this pathway. On the other hand, if rs4129267:T increases asthma risk because it increases IL-6

trans-signalling, we would expect tocilizumab to improve asthma symptoms, because it blocks sIL-6R. As previously discussed, in mice allergen-induced responses improved after tocilizumab treatment, thereby supporting the second hypothesis. Thus, a more appropriate approach to target IL-6 trans-signalling would be to use a drug such as olamkicept, which specifically blocks sIL-6R and has no effect on the classical signalling pathway.

Overall, the current study design was appropriate for a proof-of-concept assessment of the effect of a single dose of tocilizumab in asthma patients. Allergen inhalation challenge tests are routinely used to assess the efficacy of new experimental treatments for asthma. Moreover, the main efficacy endpoint that we analysed (the magnitude of the LAR after treatment) was objectively measured before and after treatment, providing the opportunity to correct for intra-individual variability that was not related to the study drug. Another advantage in our study was the recruitment of patients with the rs4129267:T allele. As discussed above, this variant is associated with increased serum levels of sIL-6R and an extensive body of evidence suggests that its blockade has anti-inflammatory effects. Our study did, however, have some limitations. First, by blocking both the mIL-6R and sIL-6R, tocilizumab may have opposing effect in airway inflammation. Second, with the current study design, we were not able to test the effect of tocilizumab in patients with more severe asthma, who are more likely to benefit from new therapies, and in particular from blockade of IL-6 signalling. Lastly, in the screening phase, only one of the 11 participants developed a mixed granulocytic inflammatory response after allergen challenge. Importantly, that patient also had the highest sputum level of IL-6 and sIL-6R, and was the only subject who did not experience a LAR after treatment with tocilizumab. We previously showed that individuals with mixed-granulocytic sputum subtype are more likely to have IL-6 trans-signalling in the airways (Chapter 2), perhaps because neutrophils are a main source of sIL-6R.[296] Accordingly, the asthma sub-group that is more likely to benefit from the anti-inflammatory effects of IL-6 trans-signalling blockade are those who also have a mixed granulocytic sputum subtype. Further studies are required to test this hypothesis.

In conclusion, we show that a single intravenous infusion of tocilizumab at a dose of 8-mg/kg does not inhibit allergen-induced airway responses in mild asthmatics. However, our results suggest that blockade of airway IL-6 trans-signalling in patients with elevated sputum levels of IL-6 and sIL-6R might provide a more promising therapeutic strategy to attenuate allergen-induced immune responses.

Chapter 7

Frequency of asthma prescriptions in patients on regular tocilizumab treatment

CHAPTER 7. FREQUENCY OF ASTHMA PRESCRIPTIONS IN PATIENTS ON REGULAR TOCILIZUMAB TREATMENT

The goal of this chapter was to assess whether long-term (i.e. 12 months) treatment with TCZ influenced the frequency of prescriptions for asthma-related medications using data from the Pharmaceutical Benefits Scheme (PBS).

Introduction

In the previous chapter, we presented results from a phase II clinical trial performed to assess the effect of single dose tocilizumab (TCZ) in the prevention of allergen-induced responses. The study design used in that study is very powerful to detect drug effects on acute exacerbations. However, it has two important limitations. First, the allergen inhalation challenge test used can only be performed in subjects with mild asthma, who at baseline have lung function within 30% of the predicted value. This ensures that allergen challenge does not cause a drop in lung function that is unsafe for the participants. As such, that study design cannot be used to assess the effect of new experimental drugs in patients who have severe asthma and, therefore, who have the greatest need for new treatments. This is particularly relevant if, for example, the pathway being targeted by the new treatment is specific to an inflammatory subtype that is common amongst patients with severe but not mild asthma. The second limitation of the clinical trial reported in the previous chapter is that it does not address the possibility that prolonged treatment with TCZ might be required to observe a clinically-significant effect on airway inflammation.

In this chapter, we conceived a study that could potentially address these limitations, at least partly. Specifically, we identified individuals who (1) were likely to have asthma, including some expected to have moderate to severe asthma symptoms, based on the number of inhaled corticosteroid prescriptions taken; and (2) were treated regularly with TCZ, which was prescribed for a different condition, most likely rheumatoid arthritis (RA). Based on co-morbidity studies, 1-7% of RA patients also suffer from asthma.[288,289,297] Thus, if TCZ has an effect on asthma – improving or worsening disease symptoms – then we hypothesised that the onset of TCZ therapy in these patients is associated with a significant change in the pattern of asthma-related prescriptions.

In Australia, information recorded in the Pharmaceutical Benefits Scheme (PBS) can be used to test this hypothesis. The PBS is a comprehensive program through which the Australian Government provides residents with affordable access to a wide range of medicines. Electronic records of claims

through the PBS are collected by the Department of Human Services, and include information on quantity, strength, and date the subsidized medicines were dispensed. Access to these data can be requested for specific research projects, thereby providing a unique opportunity to study outcomes of medicines that have been approved for particular diseases or conditions.

TCZ was approved by the Therapeutic Goods Administration for the treatment of rheumatoid arthritis (RA) in Australia in 2009. In this chapter, we analyse PBS data collected during a 5-year period to study the pattern of asthma-related prescriptions after the onset of regular TCZ treatment in individuals who were likely to suffer from both RA and asthma. Specifically, our hypothesis was that regular treatment with TCZ is associated with a decreased frequency of asthma prescriptions, reflecting an improvement in asthma symptoms.

Methods

Data source

We conducted a retrospective study of de-identified PBS individual-level data of prescription drugs subsidized by the Australian Government. Data were extracted by the Australian Institute of Health and Welfare (AIHW) for prescriptions supplied between April 2010 and March 2015, inclusive. The dataset obtained from the AIHW included six fields: (1) de-identified patient ID, (2) sex, (3) age as of 1 July 2016, (4) prescription supply date (year and month), (5) PBS item code, and (6) number of scripts issued. Four groups of drugs were included in this dataset, namely (1) TCZ, (2) inhaled corticosteroids (ICS), to identify individuals who were likely to suffer from asthma, (3) tiotropium (TIO), to exclude individuals who were likely to suffer from chronic obstructive pulmonary disease (COPD); and (4) adalimumab (ADA), a monoclonal antibody that blocks TNF-alpha and is also used to treat RA, which was used to assess if any changes in the pattern of asthma prescriptions were specific to TCZ. Figure 7.1 summarizes the number of unique individuals with prescriptions for each drug in this dataset.

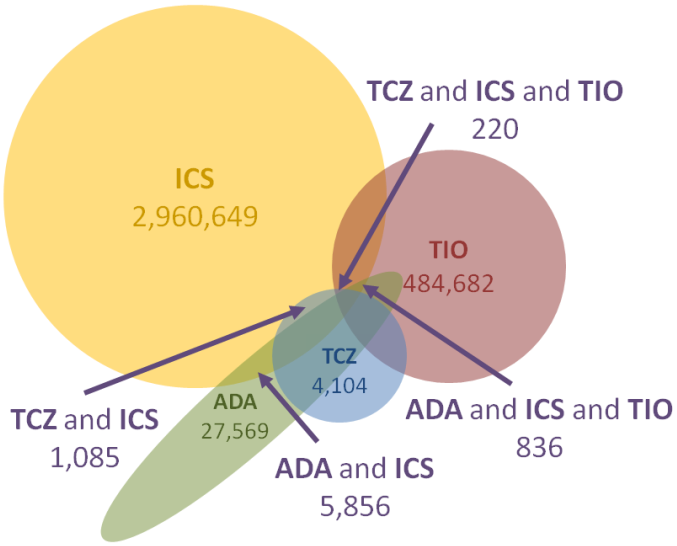


Figure 7.1. Number of unique patients with each of the four main prescription drugs analysed between April 2010 and March 2015, inclusive.

ADA, adalimumab; ICS, inhaled corticosteroids; TIO, tiotropium; TCZ, tocilizumab.

Records for all other drugs prescribed between April 2010 and June 2015 to subjects included in the dataset above were also obtained. As some items in this list were rare and potentially identifiable, these data were aggregated by the AIHW into two groups, using the Anatomical Therapeutic Chemical (ATC) classification system. Specifically, drugs for obstructive airway diseases (ATC2 code 'R03') were grouped at the ATC5 code level, and other drugs grouped at the ATC2 code level. No scripts under co-payment were included in any of the datasets. Data were exclusively derived from the PBS dataset, i.e. no linkage to other datasets was performed.

Study design

Figure 7.2 summarizes the approach used to study the effect of regular TCZ therapy on the frequency of asthma prescriptions. The number of asthma prescriptions was compared between two consecutive periods, designated as 'baseline' and 'treatment' phases. The baseline phase consisted of a 12-month period with no TCZ scripts. The treatment phase consisted of a 12-month period comprising at least 10 months with a prescription of TCZ, which is taken monthly.

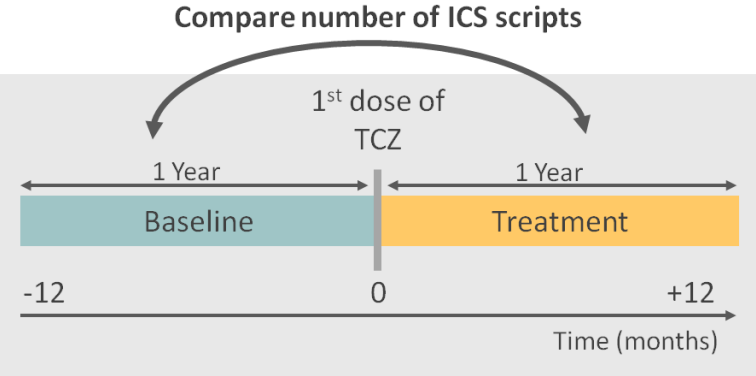


Figure 7.2. Study design

The number of ICS scripts were compared between two consecutive periods of 12 months. The first 12-month period (baseline) comprised no TCZ scripts. The second 12-month period (treatment) included at least 10 months with a TCZ script. Abbreviations: ICS, inhaled corticosteroid; TCZ, tocilizumab.

Analyses were performed in a 'case group' and in a 'control group' (specific inclusion criteria below). The case group consisted of patients treated with TCZ who were also likely to suffer from asthma. The control group consisted of subjects who were not treated with TCZ but were likely to suffer from asthma

Primary analysis

Inclusion criteria to define cases

In broad terms, we considered that an individual was likely to suffer from asthma if he/she had (1) prescriptions of ICS, which are used almost exclusively to treat asthma and COPD; and (2) no prescriptions of TIO, which is almost exclusively used to treat COPD (Table 7.1).

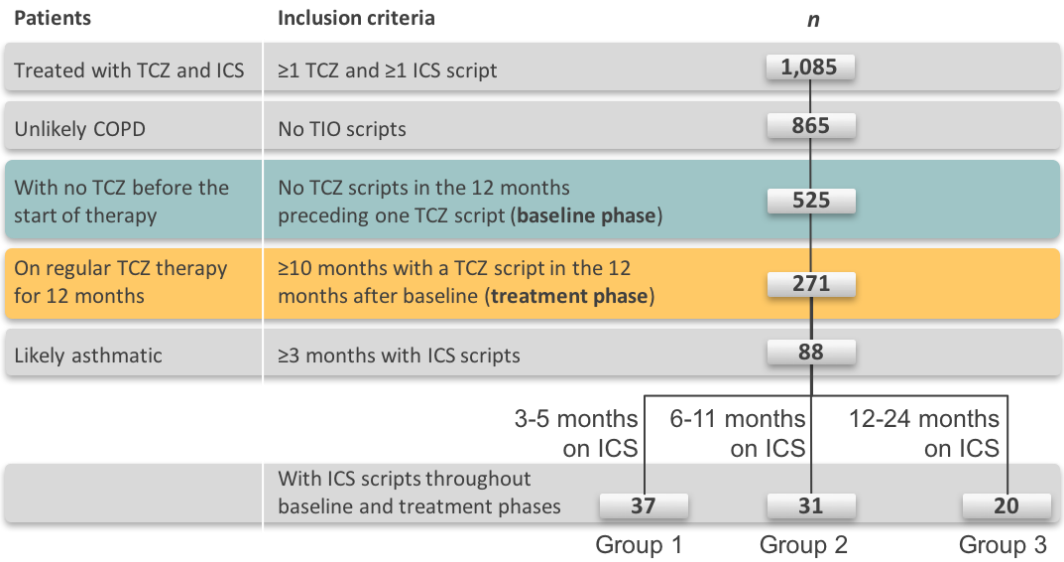


Table 7.1. Study cohort inclusion criteria.

ICS, inhaled corticosteroids; TIO, tiotropium; TCZ, tocilizumab.

More specifically, we first identified individuals who between April 2010 and March 2015 were issued (1) any number of prescriptions for TCZ; (2) any number of prescriptions for ICS; and (3) no prescriptions of TIO. Next, we identified the subset of individuals who had a 12-month period of no TCZ prescriptions (this constitutes the 'baseline phase), which was followed by a 12-month period with 10 or more months with a TCZ script ('treatment phase'). Lastly, we selected for inclusion in the 'case group' individuals with three or more ICS prescriptions during the 24-month period of analysis (i.e. baseline plus treatment phases) – this threshold was used to minimize the probability that ICS were prescribed seasonally to treat viral coughs and not necessarily asthma.

Three sub-groups of cases (Groups 1 to 3) were defined base on the frequency of ICS scripts in the 24-month period of analysis: (1) 3 to 5; (2) 6 to 11; or (3) 12 to 24 months. These groups are likely to represent individuals with different severity of disease. Demographics for individuals included in the case group are summarized in Table 7.2.

Intervention drug	Outcome drug	Group	N	Age	Sex	Number of outcome prescriptions
TCZ	ICS	*All*	88	61, 11.1, 27-84	81.8	10, 6.7, 3-28
		Group 1	37	58, 10.6, 32-81	75.7	4, 0.9, 3-6
		Group 2	31	62, 10.3, 41-82	87.1	9, 3.7, 6-22
		Group 3	20	64, 12.7, 27-84	85	20, 4.3, 13-28
	Salbutamol	*All*	49	61, 12, 27-84	85.7	8, 6, 3-33

		Group 1	22	63, 12.5, 32-82	77.3	4, 2.1, 3-12
		Group 2	19	58, 10.7, 27-73	94.7	9, 3.4, 6-20
		Group 3	8	65, 13.3, 47-84	87.5	18, 6.8, 12-33
	OCS	*All*	422	59, 14.2, 5-92	76.1	14, 9.6, 3-58
		Group 1	122	55, 15.2, 5-92	76.2	5, 2.3, 3-15
		Group 2	131	60, 13.2, 7-89	77.1	11, 3.6, 6-22
		Group 3	169	62, 13.7, 19-88	75.1	23, 8, 12-58
ADA	ICS	*All*	272	54, 15.2, 5-85	69.5	9, 6.4, 3-45
		Group 1	110	53, 15.3, 16-78	70	4, 1.7, 3-13
		Group 2	106	54, 15.9, 5-85	70.8	9, 2.6, 6-19
		Group 3	56	56, 13.9, 23-79	66.1	19, 6.5, 12-45
	Salbutamol	*All*	141	54, 14.3, 2-78	70.9	9, 7.2, 3-42
		Group 1	72	57, 12.6, 22-78	70.8	4, 1.5, 3-10
		Group 2	46	52, 14.4, 5-75	73.9	10, 2.6, 6-18
		Group 3	23	50, 17.7, 2-76	65.2	22, 8, 12-42
	OCS	*All*	942	55, 17.2, 3-89	67.6	12, 8.1, 3-62
		Group 1	345	51, 18.4, 4-89	65.8	5, 2.2, 3-21
		Group 2	330	55, 17.5, 3-87	67	11, 3.6, 6-30
		Group 3	267	61, 13.1, 18-85	70.8	21, 7.7, 12-62
ICS	Salbutamol	*All*	3088	57, 24, 0-103	58.1	12, 12, 3-297
		Group 1	1027	59, 23.2, 0-98	62.1	4, 1.6, 3-31
		Group 2	1278	55, 24.9, 0-103	57.4	10, 4.2, 6-58
		Group 3	783	57, 23.4, 0-102	54	25, 16.8, 12-297
OCS	ICS	*All*	849	68, 15.8, 0-100	59.4	10, 7.1, 3-44
		Group 1	313	67, 15.6, 0-100	56.9	4, 1.7, 3-26
		Group 2	292	67, 16.5, 1-98	59.2	10, 3.3, 6-35
		Group 3	244	70, 14.9, 8-100	62.7	19, 5.4, 12-44

Table 7.2. Demographics of cases studied.

Age and sex were assessed at the day of the first prescription of the intervention drug. The number of outcome prescriptions represents the total number of prescriptions observed in the 24-month period of analysis. Matched controls had the same demographics but had no prescriptions for the intervention drug. Age and number of outcome prescriptions are presented as mean, standard deviation and range. Sex is presented as percentage of female. ICS, inhaled corticosteroids; OCS, oral corticosteroids; TCZ, tocilizumab.

Inclusion criteria to define controls

To create a group of controls for the TCZ analysis, we selected individuals who were likely to suffer from asthma but who were not treated with TCZ. We identified up to five matched controls for each case. Controls were matched on age, sex and number of ICS scripts issued during the same 24-month period of analysis. For example, a male who (1) was born in 1958, (2) started regular TCZ therapy in

January 2014, and (2) had nine ICS prescriptions from January 2013 to January 2015, was matched with up to five males born in 1958, who had nine ICS prescriptions between January 2013 and January 2015, but none of TCZ.

Secondary analyses

Effect of TCZ on other asthma medications

We conducted secondary analyses with the same analytical approach used in the primary analysis, but defined cases based on different outcome drugs.

First, we identified a group of cases with the exact same selection criteria as before, but the outcome drug was salbutamol instead of ICS, i.e. we selected individuals with (1) ≥ 3 months with salbutamol prescriptions in the 24 months analysed, (2) no tiotropium prescriptions ever, and (3) a period of 12 months with no TCZ (baseline), followed by a period of 12 months with ≥ 10 TCZ prescriptions (treatment). We identified 49 individuals with these characteristics, and defined three sub-groups based on the frequency of salbutamol prescriptions, as before.

Second, we identified a group of cases with (1) ≥ 3 months with OCS prescriptions in the 24 months analysed, (2) no tiotropium prescriptions ever, and (3) a period of 12 months with no TCZ (baseline), followed by a period of 12 months with ≥ 10 TCZ prescriptions (treatment). A total of 942 individuals met these criteria and were divided into three sub-groups based on the frequency of OCS prescriptions.

These groups were used to compare the number of salbutamol or OCS prescriptions before and after the onset of regular TCZ therapy. Matched controls were defined with the exact same criteria and approach described in the primary analysis.

Demographics for individuals included in these analyses are summarized in Table 7.2.

Effect of ADA on ICS or other asthma medications

To assess if TCZ-associated changes in the pattern of asthma prescriptions were specific to TCZ, we performed the same analyses as before (i.e. with ICS, salbutamol or OCS as the outcome drug) but selected cases that were treated with ADA instead of TCZ. A matched control group was also identified in each analysis using the exact same criteria and approach described above. Demographics for individuals included in these analyses are summarized in Table 7.2.

Effect of strong asthma medications on other asthma medications

Lastly, we evaluated the ability of our study design to identify changes in the number of asthma prescriptions, based on the assumption that stronger asthma medications (e.g. ICS), which help control asthma, may result in a decrease in the number of other asthma prescriptions (e.g. salbutamol). Specifically, we performed two analyses. First, we compared the number of salbutamol prescriptions before and after the onset of ICS therapy. Second, we compared the number of ICS prescriptions before and after the onset of OCS therapy. Cases were defined with the same criteria used in the primary analysis, except that the intervention (TCZ) and outcome (ICS) drugs were respectively replaced by ICS and salbutamol in the first analysis, and OCS and ICS in the second analysis. In both analyses matched controls were defined with the exact same criteria as before. Demographics for individuals included in these analyses are summarized in Table 7.2.

Statistical analyses

A Wilcoxon signed rank test was used to compare (1) the number of asthma prescriptions between the baseline and treatment phases, and (2) the number of asthma prescriptions issued at baseline between the case and control groups. Analyses were conducted using R version 3.4.1.

Results

Frequency of asthma prescriptions before and after the start of TCZ therapy

Between April 2010 and March 2015, a total of 132,780 TCZ prescriptions were issued to 4,104 unique individuals. Of these, 88 (2.1%) were included in the 'case group' because they (1) were prescribed TCZ for at least 10 of 12 consecutive months; (2) in the preceding 12 months had no TCZ prescription; (3) had three or more ICS prescriptions in the combined 24-month period; and (4) had no TIO prescriptions in the 5-year period. The 88 individuals were then divided into three case sub-groups according to the number of months with an ICS prescription across the 24-month period: (1) 3 – 5 months (Group 1; $n = 37$); (2) 6 – 11 months (Group 2; $n = 31$); and (3) 12 – 24 months (Group 3; $n = 20$).

First, for each case group, we compared the frequency of ICS prescriptions between the baseline and treatment phases, that is, before and after the onset of regular TCZ treatment. We found that the number of ICS prescriptions taken in the 12 months that followed the onset of TCZ therapy was comparable to that observed in the preceding 12 months without TCZ treatment (Figure 7.3 A). This indicates that the pattern of ICS use did not significantly change in these individuals after the onset

of TCZ therapy. To help visualize this, for each month, we estimated the proportion of individuals with an ICS prescription, and plotted these estimates across the 24-month period considered (Figure 7.3 B). For example, in Group 3 cases (i.e. those with this greatest number of ICS prescriptions over the 24-month period; *n* = 20), 70% of individuals had an ICS prescription on average per month in the baseline period, and this increased to 73% in the treatment period. These figures were comparable to what was observed in the matched control group (69% vs. 74%; *n* = 100), indicating that the small increase in proportion of individuals taking ICS observed in the treatment phase was unlikely to be related to TCZ treatment. Broadly similar results were observed in Group 1 and 2 cases.

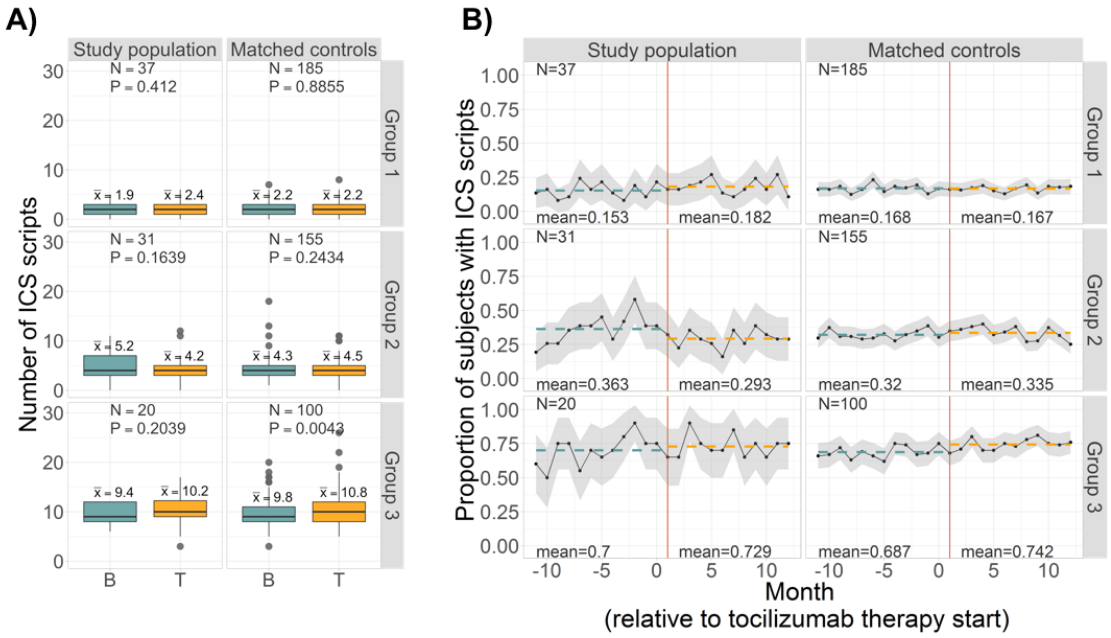


Figure 7.3. Number of ICS prescriptions (A) and proportion of patients with ICS prescriptions (B) before and after the start of regular TCZ therapy.

Dashed lines represent the mean. Light grey shading represents the 95% margin of error of proportions. Red vertical line indicates date of first TCZ script. Group 1, 3-5 months with ICS; Group 2, 6-11 months with ICS; Group 3, 12-24 months with ICS. B, baseline phase; ICS, inhaled corticosteroids; T, treatment phase.

Next, we used the exact same approach to assess the impact of regular TCZ therapy on the frequency of two other asthma medications: salbutamol and oral corticosteroids (OCS). We found that the onset of TCZ therapy did not have a significant effect on salbutamol use (Figure 7.4). Because salbutamol is used almost exclusively to treat asthma, these results suggest that TCZ therapy did not have a significant effect on asthma symptoms, as observed for ICS.

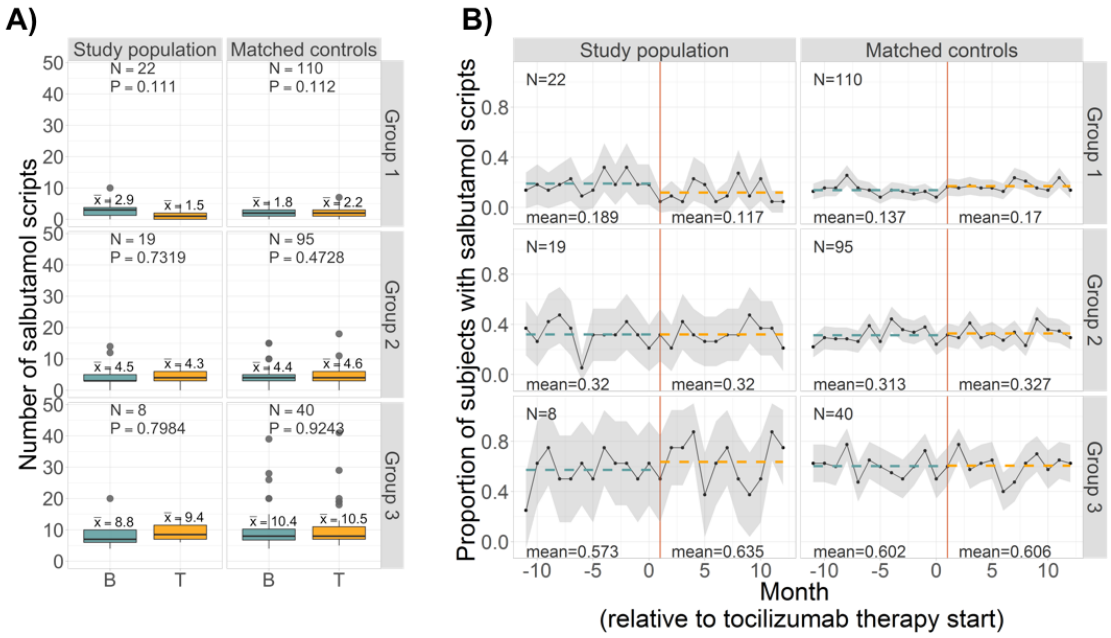


Figure 7.4. Number of salbutamol prescriptions (A) and proportion of patients with salbutamol prescriptions (B) before and after the start of regular TCZ therapy.

Dashed lines represent the mean. Light grey shading represents the 95% margin of error of proportions. Red vertical line indicates date of first TCZ script. Group 1, 3-5 months with salbutamol; Group 2, 6-11 months with salbutamol; Group 3, 12-24 months with salbutamol. B, baseline phase; T, treatment phase.

On the other hand, we found the number of OCS prescriptions dropped significantly ($P = 5.9 \times 10^{-7}$) in the 12 months that followed the onset of TCZ therapy in the case group that had the lowest overall number of OCS prescriptions (Figure 7.5 A). This was a reflection of a reduction in the proportion of patients taking OCS prescriptions, from 22% at baseline to 10% in the treatment phase (Figure 7.5 B). This pattern of change was not observed in the matched controls (Figure 7.5 A and B), indicating that it was indeed related to the onset of TCZ treatment. A similar trend was observed in those with intermediate (Group 2; $P = 0.03$) but not highest (Group 3; $P = 0.83$) overall use of OCS. These results indicate that in patients with a low to intermediate number of OCS scripts (possibly prescribed for the treatment of RA), the onset of TCZ therapy is associated with a reduction in OCS intake.

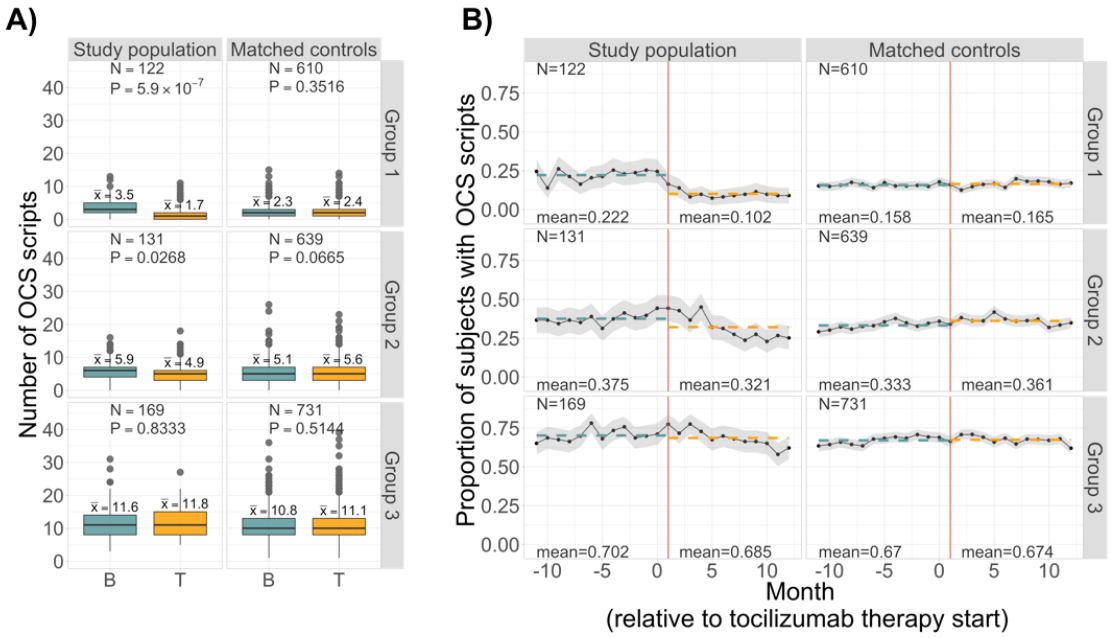


Figure 7.5. Number of OCS prescriptions (A) and proportion of patients with OCS prescriptions (B) before and after the start of regular TCZ therapy.

Dashed lines represent the mean. Light grey shading represents the 95% margin of error of proportions. Red vertical line indicates date of first TCZ script. Group 1, 3-5 months with OCS; Group 2, 6-11 months with OCS; Group 3, 12-24 months with OCS. B, baseline phase; OCS, oral corticosteroids; T, treatment phase.

Frequency of asthma prescriptions before and after the start of adalimumab therapy

To determine the extent to which the results above were specific to TCZ therapy, we analysed data from subjects who started regular treatment with adalimumab (ADA) – another monoclonal antibody used to treat RA – instead of TCZ.

We found that the effect of ADA treatment on the frequency of ICS (Figure 7.6), salbutamol (Figure 7.7) and OCS (Figure 7.8) was very similar to that observed with TCZ. The only exception was that OCS prescriptions decreased during the treatment phase in all three case groups, including those with highest use of OCS (Group3). Thus, as for TCZ, our results suggest that ADA therapy results in a significant improvement in RA symptoms which prompts the reduction in OCS prescriptions. The lack of a significant effect on the frequency of medications that are typically used to treat asthma (ICS and salbutamol) suggests that ADA too (as TCZ) does not significantly influence asthma symptoms.

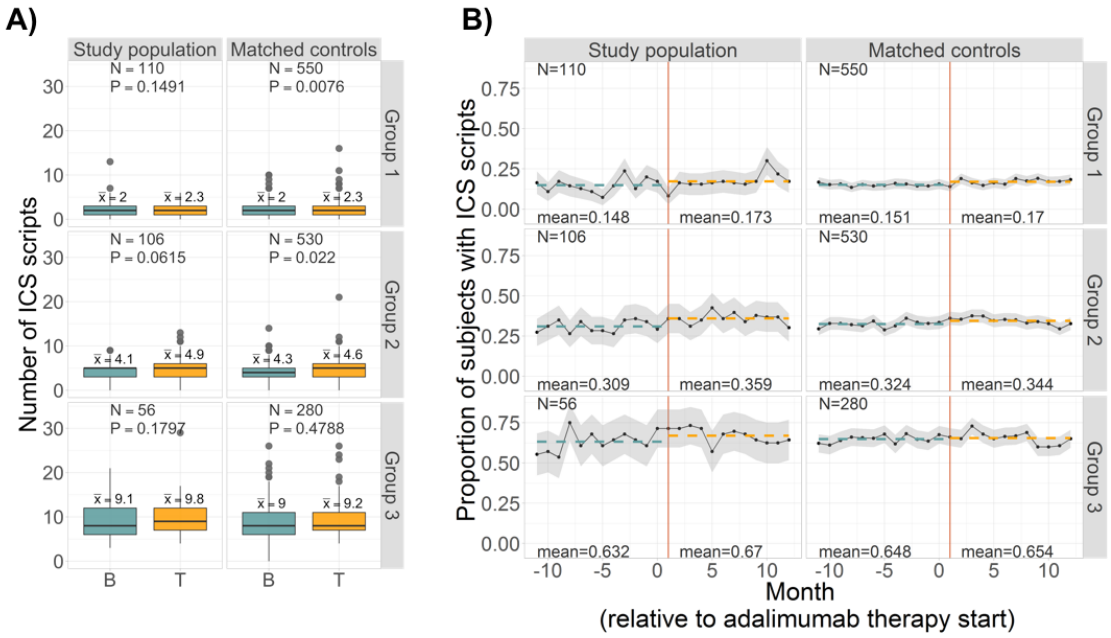


Figure 7.6. Number of ICS prescriptions (A) and proportion of patients with ICS prescriptions (B) before and after the start of regular adalimumab therapy.

Dashed lines represent the mean. Light grey shading represents the 95% margin of error of proportions. Red vertical line indicates date of first adalimumab script. Group 1, 3-5 months with ICS; Group 2, 6-11 months with ICS; Group 3, 12-24 months with ICS. B, baseline phase; ICS, inhaled corticosteroids; T, treatment phase.

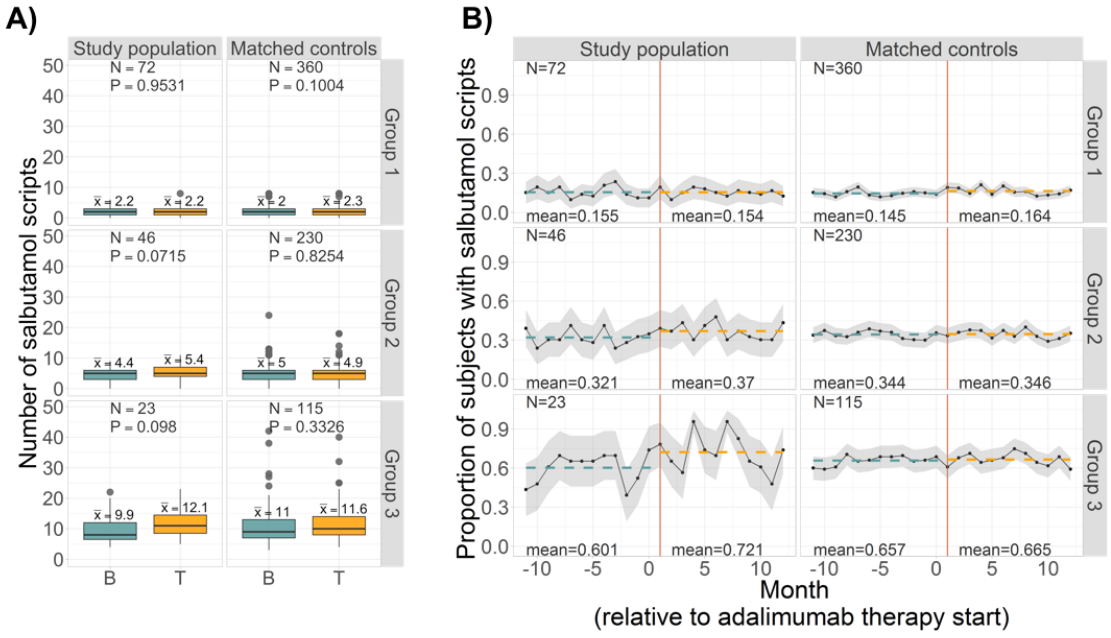


Figure 7.7. Number of salbutamol prescriptions (A) and proportion of patients with salbutamol prescriptions (B) before and after the start of regular adalimumab therapy.

Dashed lines represent the mean. Light grey shading represents the 95% margin of error of proportions. Red vertical line indicates date of first adalimumab script. Group 1, 3-5 months with salbutamol; Group 2, 6-11 months with salbutamol; Group 3, 12-24 months with salbutamol. B, baseline phase; T, treatment phase.

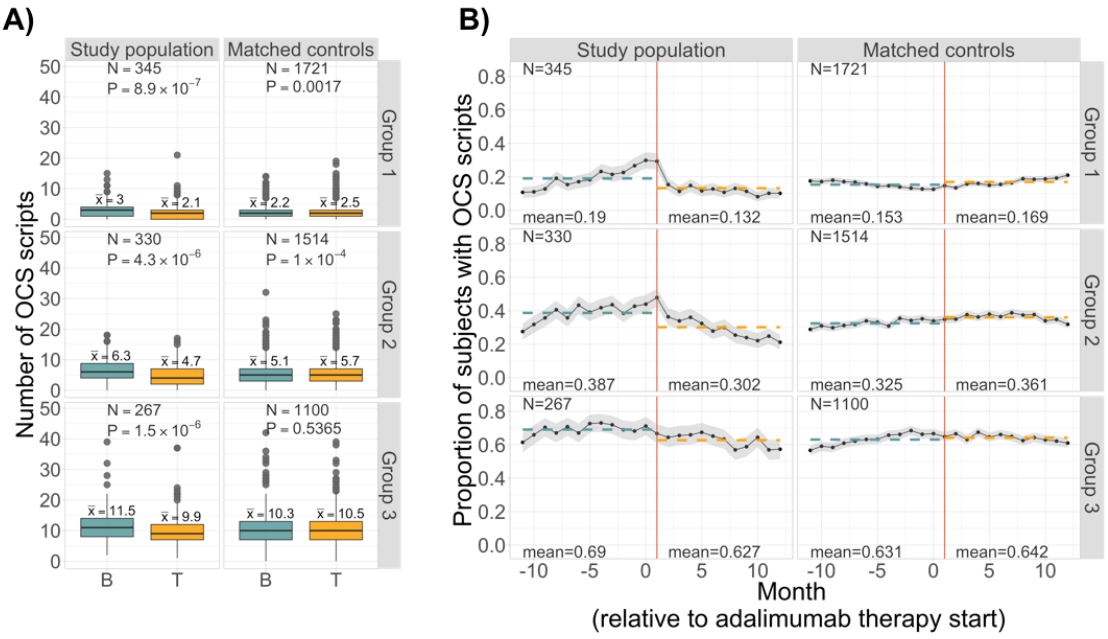


Figure 7.8. Number of OCS prescriptions (A) and proportion of patients with OCS prescriptions (B) before and after the start of regular adalimumab therapy.
Dashed lines represent the mean. Light grey shading represents the 95% margin of error of proportions. Red vertical line indicates date of first adalimumab script. Group 1, 3-5 months with OCS; Group 2, 6-11 months with OCS; Group 3, 12-24 months with OCS. B, baseline phase; OCS, oral corticosteroids; T, treatment phase.

Frequency of asthma prescriptions before and after the start of therapy with a stronger asthma medication

Lastly, we determined if our study design was indeed able to identify a change in the frequency of asthma-related prescriptions when such an effect was expected. To this end, we analysed the frequency of prescriptions for a particular asthma treatment (e.g. salbutamol) before and after the onset of stronger asthma treatment (e.g. ICS). For example, after the start of ICS, which help prevent asthma symptoms, we hypothesised that the frequency of salbutamol prescriptions would decrease.

Unexpectedly, we observed that the number of salbutamol prescriptions increased markedly after the start of ICS treatment, in all three case groups considered (Figure 7.9 A). For example, in Group 1,

the proportion of patients with salbutamol scripts per month increased from 8% to 25%, whereas marginal increases (16% to 17%) were observed in matched controls (Figure 7.9 B). Notably, the number of salbutamol prescriptions was consistently higher in the baseline phase in matched controls, who by definition did not start ICS treatment (Table 7.3). A similar pattern of results was observed when we assessed the effect of OCS therapy on the frequency of ICS prescriptions (Figure 7.10 A). Possible explanations for this unexpected finding are discussed below.

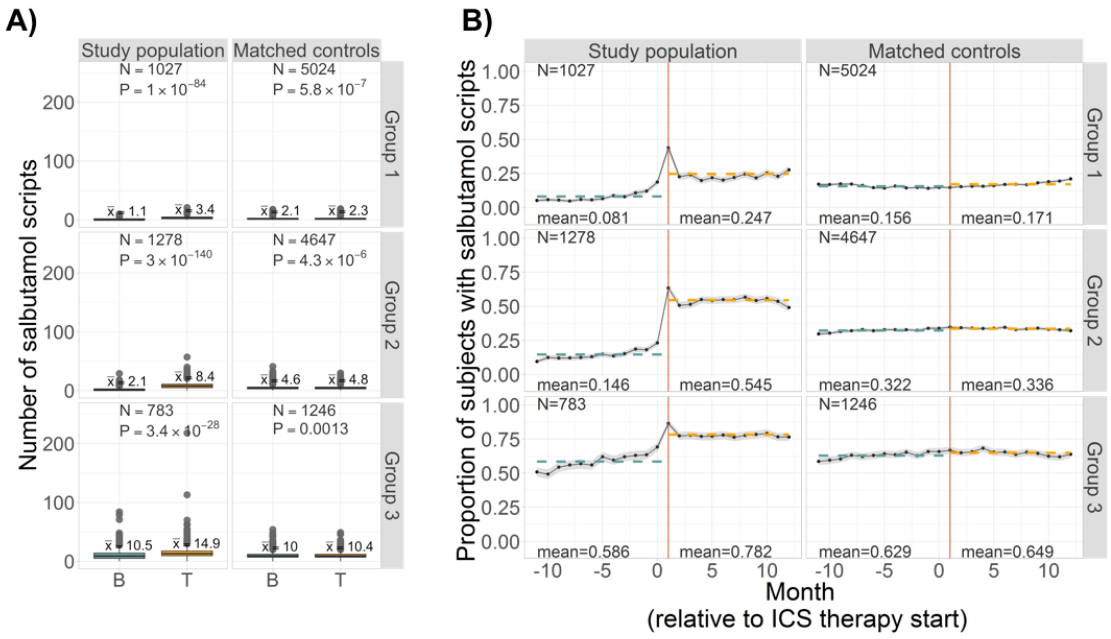


Figure 7.9. Number of salbutamol prescriptions (A) and proportion of patients with salbutamol prescriptions (B) before and after the start of regular ICS therapy.

Dashed lines represent the mean. Light grey shading represents the 95% margin of error of proportions. Red vertical line indicates date of first ICS script. Group 1, 3-5 months with salbutamol; Group 2, 6-11 months with salbutamol; Group 3, 12-24 months with salbutamol. B, baseline phase; ICS, inhaled corticosteroids; T, treatment phase.

			Number or prescriptions in baseline phase		
Intervention drug	Outcome drug	Group	Cases	Matched controls	*P*-value
TCZ	ICS	1	1.89	2.17	0.3572
		2	5.23	4.32	0.2251
		3	9.45	9.76	0.8507
	Salbutamol	1	2.86	1.81	0.0417
		2	4.53	4.41	0.4338
		3	8.75	10.43	0.4415
	OCS	1	3.48	2.31	9×10^{-10}

		2	5.88	5.11	0.0100
		3	11.63	10.83	0.0572
ADA	ICS	1	2.02	2.02	0.7636
		2	4.12	4.27	0.8018
		3	9.11	8.96	0.7854
	Salbutamol	1	2.15	2.01	0.4735
		2	4.37	4.98	0.3738
		3	9.87	11.02	0.3696
	OCS	1	3.01	2.2	2×10^{-12}
		2	6.26	5.06	3×10^{-9}
		3	11.46	10.29	0.0001
ICS	Salbutamol	1	1.07	2.15	1×10^{-89}
		2	2.07	4.55	8×10^{-195}
		3	10.46	10.03	0.5854
OCS	ICS	1	1.88	2.14	0.0216
		2	4.08	4.66	0.0001
		3	9.27	9.5	0.8662

Table 7.3. Comparison of average number of prescriptions in the baseline phase, between cases and matched controls.

Group 1, 3-5 months with outcome drug; Group 2, 6-11 months with outcome drug; Group 3, 12-24 months with outcome drug. ADA, adalimumab; ICS, inhaled corticosteroids; OCS, oral corticosteroids; TCZ, tocilizumab.

Discussion

In the current study, we used a nation-wide database of medical prescriptions to analyse the effect of long-term exposure to TCZ on the frequency of asthma prescriptions, as an indirect measure of asthma symptoms.

ICS were prioritized as the main outcome in our analyses, as this class of drugs is primarily used to treat asthma and COPD patients. To enrich our case group for patients with asthma, we excluded patients who had ever been prescribed tiotropium, a drug specifically used for the treatment of COPD. We identified 88 patients who started regular TCZ therapy and who were regularly prescribed ICS. In this cohort, there were no differences in the frequency of ICS use before and after TCZ onset. As discussed in the previous chapter, this may be a consequence of the administration route currently used to deliver TCZ, which may not be adequate to target the airways, and consequently, might not provide symptom relieve.

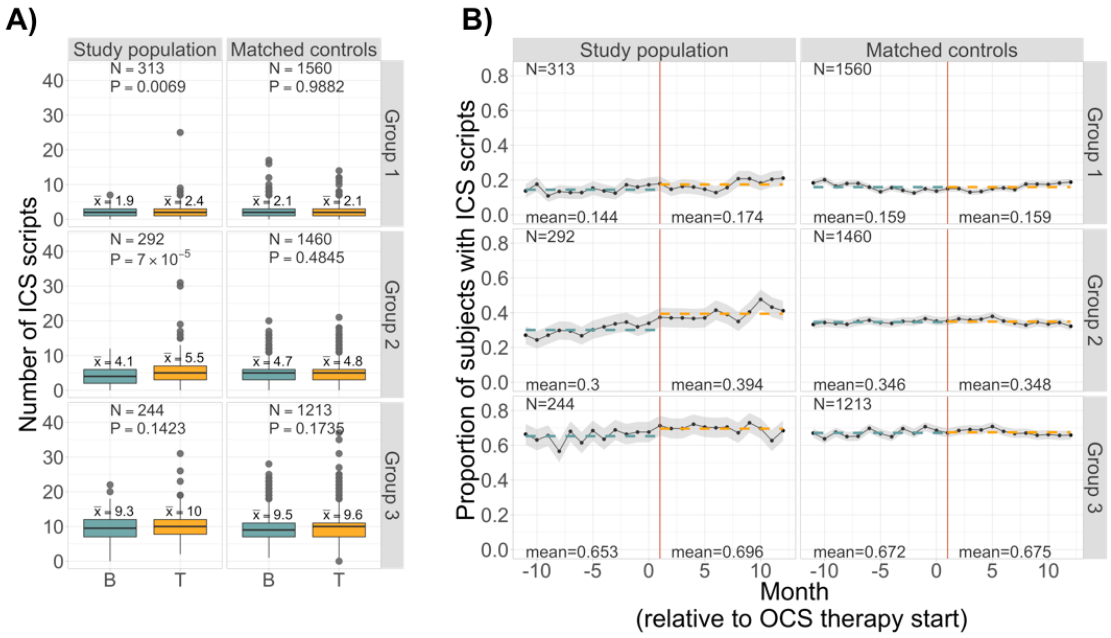


Figure 7.10. Number of ICS prescriptions (A) and proportion of patients with ICS prescriptions (B) before and after the start of regular OCS therapy.

Dashed lines represent the mean. Light grey shading represents the 95% margin of error of proportions. Red vertical line indicates date of first OCS script. Group 1, 3-5 months with ICS; Group 2, 6-11 months with ICS; Group 3, 12-24 months with ICS. B, baseline phase; ICS, inhaled corticosteroids; OCS, oral corticosteroids; T, treatment phase.

On the other hand, we observed a significant decrease in oral steroid use after the start of TCZ treatment. OCS are anti-inflammatory drugs that are prescribed to asthma patients with severe, hard-to-control symptoms. However, OCS are also used to treat other conditions such as arthritis, severe allergies and skin conditions. Thus, unlike ICS, these data could potentially represent treatments for conditions other than asthma. Indeed, previous studies that evaluated the efficacy of TCZ in various forms of arthritis, including RA and systemic juvenile idiopathic arthritis (two conditions that are currently treated with TCZ), have shown that OCS use decreases in patients treated with TCZ.[298-300] Thus, it is likely that the decreases in number of OCS prescriptions that we observed in our study corresponded to a decrease in OCS use by arthritis patients who were OCS-users and became less dependent on these drugs after TCZ onset. This is further corroborated by the observation that the start of therapy with ADA – another drug used to treat arthritis – was also accompanied by a decreased in OCS use.

The study design used here was able to detect plausible changes in the frequency of use of arthritis treatments after the start of TCZ and ADA – two biologic agents used to treat more severe cases of disease. To determine whether similar changes would be observed in asthma treatment plans after the introduction of stronger asthma medications, we also assessed the patterns of salbutamol use before and after the start of ICS treatment. ICS are preventer drugs used to control asthma symptoms. Typically, these medications are prescribed as-needed or when regular use of reliever medications is not sufficient to control asthma symptoms. We expected ICS onset to result in an improvement in symptom control and, consequently, to be associated with a decrease in the use of reliever drugs, such as salbutamol. Instead, we observed a large increase in the frequency of salbutamol prescriptions after the start of regular ICS treatment. Interestingly, these increases were not observed in matched controls, who had higher rates of ICS use in the baseline phase. These observations suggest that, in controls, regular use of reliever medication contributed to well-controlled asthma, and consequently no step-up was required in their treatment plan. This hypothesis is corroborated by extensive evidence that treatment adherence is associated with a decreased frequency of asthma symptoms.[301-304] On the other hand, patients who required adding ICS to their treatment plan were likely to have experienced a worsening of symptoms due to poor-controlled asthma, in which case medical advice would have been sought. This hypothesis is supported by the fact that the increase in salbutamol use started before ICS onset. On the other hand, the sharp increase in salbutamol use coinciding with the first ICS dose, suggests that the use of reliever medication was part of a new treatment plan outlined by a clinician. Another possibility is that, when compared to matched controls, individuals who started ICS treatment were more likely to be asymptomatic (rather than have poorly-controlled symptoms) during the baseline period.

An appropriate negative control for our analyses would have been a group of individuals not likely to have asthma but with records of asthma prescriptions (e.g. ICS). This would allow us to assess the effect of TCZ on the frequency of asthma-related prescriptions. Our approach to select asthma patients was to select individuals treated with asthma prescriptions and exclude individuals with tiotropium prescriptions, as those are exclusively prescribed to COPD patients. Thus, using the same criteria, a control group taking asthma prescriptions and TCZ but not likely to have asthma would include the subjects with tiotropium prescriptions (not exclude them), i.e. COPD patients. Unfortunately, this is not a good negative control because, as for asthma, there is compelling evidence that the IL-6 trans-signalling pathway may contribute to the pathophysiology of COPD.[256,305,306] Thus,

TCZ could potentially have the same effects in the use of medications like ICS in the group of 'likely asthmatics' and the group of 'likely non-asthmatics'.

Instead, our negative control were individuals that were likely to be asthmatics and who started regular therapy with ADA (instead of TCZ). We recognise that this is not a perfect negative control. Single-case reports have provided evidence of asthma-like symptoms in RA patients after the onset of ADA therapy.[307-309] Thus, asthma may be an adverse event of ADA therapy. Importantly, the patients in these reports were treated with asthma medications, including ICS and salbutamol, so if our analyses included cases like these our results could be biased. Nevertheless, ADA was tested in thousands of patients with different conditions (including RA, juvenile idiopathic arthritis, psoriatic arthritis, psoriasis, ulcerative colitis, ankylosing spondylitis, and Crohn's disease) and asthma was not a common adverse event in those studies.[310] In RA clinical trials, common adverse events reported (> 10% incidence) included infections, injection site reactions, headaches and rashes.[311-315] More importantly, the incidence at which asthma-related symptoms were reported in these studies was less than 5%.[316] Thus, we performed our analyses based on the assumption that the low incidence of these cases would not affect our results. We did not observe any increases in asthma prescriptions after the onset of adalimumab therapy. Thus, it is unlikely that cases like the ones mentioned above biased our results.

A few considerations need to be taken when interpreting the results of this study. First, it is important to note that the results presented here are exclusively based on medical prescription data. Thus, case ascertainment may not be as specific as if diagnostic information were available. In addition, when assessing the changes in medical prescriptions, it is important to note that prescription data may not be an exact representation of drug use. Second, the study design that we used is more likely to detect upgrades in asthma treatment than downgrades or no changes, as patients are more likely to visit the doctor (and so to be prescribed ICS or OCS) when their symptoms worsen. In addition, while clinicians aim to prescribe the lowest doses required to control patient symptoms, there is typically greater caution when reducing treatment. Nevertheless, the results obtained for OCS use suggest that this study design can be used to monitor long-term drug effects on the condition they were approved for.

In conclusion, our results suggest that long-term treatment with TCZ does not improve (or worsen) asthma symptoms, assessed through the number and frequency of use of ICS and salbutamol prescriptions.

Conclusion

CONCLUSION

The overall goal of this dissertation was to evaluate the role of the IL-6 signalling pathway in asthma and to evaluate whether its blockade represents a potential new treatment option for asthma. Specifically, four main questions were addressed:

1. What characterises asthma patients who are likely to benefit from anti-IL-6R therapy?
2. Is IL-6 trans-signalling more likely to be promoted by some allergen types?
3. Are there other genes that associate with IL-6 signalling that are also risk factors for asthma?
4. Can asthma symptoms be attenuated by a drug that blocks IL-6 signalling?

This section presents a synthesis of how each research question was addressed and outlines the main findings and implications of each study.

Chapter 2: What characterises asthma patients who are likely to benefit from anti-IL-6R therapy?

Overview: Previous studies suggested that blockade of the IL-6 pathway could represent a new treatment option for asthma. Tocilizumab – a drug that blocks IL-6R – was already approved to treat a different condition, and so represented the ideal candidate to test this hypothesis in a phase IIA (i.e. proof-of-concept) clinical trial of asthma. Phase IIA trials are designed to assess the efficacy of a given drug in a selected disease or condition. Typically, these studies have small sample sizes to reduce patient exposure to unexpected drugs effects that might be specific (or more common) in a patient group for which the drug was not developed for. It is at this stage of drug development that most drugs fail to show a clinically significant effect in the disease that they were originally designed for.[317,318] An approach that can increase the success rate of phase II trials is to test drugs in a selected group of patients who are most likely to respond to the new intervention. The first aim of this thesis was to identify the group of asthmatics most likely to benefit from anti-IL-6R therapy, as this information could then help design subsequent phase II trials of tocilizumab. A key strength of this study was the use of a combined phenotype that integrated information from both IL-6 and sIL-6R levels (the two key components of IL-6 trans-signalling) measured in sputum. As previously noted, using multivariate approaches and well defined phenotypes is essential to identify the subsets of asthma patients that are more likely to respond to specific treatments.

Main findings and conclusions: High levels of IL-6 and sIL-6R in sputum were observed more frequently in asthmatics with neutrophilic or mixed granulocytic airway inflammation. Therefore, asthma exacerbations are likely to include activation of IL-6 trans-signalling in patients with a sputum subtype that is rich in neutrophils. Sputum neutrophilia is associated with more severe symptoms,[139,319] and so anti-IL-6R treatments might be useful to treat patients who have the greatest need for new therapies. The small sample size of the cohort analysed, and the potential effect of DTT in sputum cytokine measurements are caveats of this study. Nevertheless, our results are in line with recent observations that patients with a gene signature of IL-6 trans-signalling activation in the airways produce sputum rich in eosinophils and neutrophils.[134]

Future directions: The association between neutrophil-rich airway inflammation and IL-6 trans-signalling should be validated in patients with severe asthma. Future studies are also needed to identify biomarkers of IL-6 trans-signalling in the airways.

Chapter 3: Is IL-6 trans-signalling more likely to be promoted by some allergen types?

Overview: Mice exposed to cockroach but not house-dust mite (HDM) allergens develop airway inflammation that includes neutrophilia and activation of IL-6 trans-signalling.[33] In this chapter, we tested if this observation might also extend to humans. Specifically, we compared the frequency of sputum inflammatory subtypes between asthmatics (n = 129) exposed to different allergens in inhalation challenge tests performed in previous clinical trials. Five allergen types were represented: the house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, ragweed, grass, and cat. The breadth of allergens tested and the reasonable sample size in which each one was tested is a key strength of this study.

Main findings and conclusions: We found no significant differences in the frequency of sputum inflammatory subtypes between different allergen types. Thus, unlike reported in mice, our findings indicate that in humans the sputum inflammatory subtype observed after an allergen-induced asthma exacerbation is unlikely to be influenced by the type of allergen use. Together with our previous findings (Chapter 2), these results suggest that activation of IL-6 trans-signalling in human airways is not triggered more frequently by a specific allergen type.

Future directions: A major caveat of this study was that sputum levels of IL-6 and sIL-6R were not measured in the original clinical trials. This can be addressed in future studies that directly test whether airway IL-6 trans-signalling is associated with exposure to different allergens. Ideally, these

studies should be based on a repeated-measures design to minimize the effect of potential confounders; that is, IL-6 and sIL-6R levels should be measured in the same individuals after exposure to different allergens.

Chapters 4 and 5: Are there other genes that associate with IL-6 signalling that are also risk factors for asthma?

Overview: Despite extensive evidence supporting the involvement of the IL-6 signalling pathway in asthma, little was known about the extent to which the IL-6 pathway also included other genes whose expression might also have a causal role in disease pathophysiology. In Chapters 4 and 5 we tested the hypothesis that genes related to IL-6 signalling (other than *IL6R*) also harbour risk variants for asthma. Specifically, we first searched for genes that satisfied two criteria (Chapter 4): (i) gene expression associated with *IL6R* expression; and (ii) gene expression associated with a SNP that was also associated with asthma risk. We then studied the association between a SNP of interest and asthma risk in an independent study (Chapter 5). A key strength these analyses were the different levels of evidence that linked the genes to asthma and the IL-6 signalling. Namely, genes expression levels (measured across different cell types and different individuals), eQTL studies and three independent asthma GWAS.

Main findings and conclusions: We identified five genes that satisfied both criteria: *IL18R1*, *IL18RAP*, *BCL6*, *STAT6* and *STOML2* (Chapter 4). Of these, the latter represents a potential new player in asthma pathophysiology, with a known role in T-cell activation. We noted that $CD4^+$ T cells of healthy individuals have high expression of *IL6R* and low expression of *STOML2*. On the other hand, SNPs that increased the expression of *STOML2* were associated with an increased risk of asthma and hay fever, most notably amongst individuals with early disease onset (Chapter 5). Based on these observations, we postulated that high expression of both *IL6R* and *STOML2* might result in stronger T-cell responses to allergens, thereby increasing asthma risk.

Future directions: Functional studies are required to (1) validate the contribution of *STOML2* to IL-6 signalling; and (2) test our prediction that high expression of both *IL6R* and *STOML2* results in stronger T-cell responses.

Chapters 6 and 7: Can asthma symptoms be attenuated by a drug that blocks IL-6 signalling?

Overview: The final question addressed in this thesis was whether blockade of the IL-6 signalling pathway with tocilizumab could potentially attenuate asthma symptoms. First, we analysed results

from a clinical trial that evaluated the safety and efficacy of a single dose of tocilizumab in allergen-induced responses. Eleven patients with mild allergic asthma were randomized to receive an intravenous infusion of either tocilizumab (n = 6) or placebo (n = 5). Participants performed an allergen inhalation challenge test before and after infusion. Then, allergen-induced responses were compared before and after tocilizumab. Second, we analysed data from the Pharmaceutical Benefits Scheme (PBS) to assess the effect of regular tocilizumab treatment on the frequency of asthma-related prescriptions, the latter used as a proxy for the severity of asthma symptoms.

Main findings and conclusions: Results from the clinical trial showed that tocilizumab was well tolerated but did not significantly attenuate allergen-induced bronchoconstriction. However, of note, the only individual who did not develop a late asthmatic response after treatment was in the tocilizumab group, had the rs4129267:TT genotype, mixed granulocytic sputum, and high sputum levels of IL-6 and sIL-6R. The latter observation broadly supports findings from previous chapters that anti-IL-6R therapy might be effective in attenuating an inflammatory response that includes activation of IL-6 trans-signalling. The study design used is particularly strong as it used screening measurements to correct for intra-individual variability that is unrelated to the investigational drug, and a placebo control group to distinguish drug-specific effects. On the other hand, the study was limited by the drug selected to block IL-6 signalling (tocilizumab), as it blocks two pathways (classical and trans-signalling pathway) that may have opposing effects in inflammatory responses.[32,33,40,41] In addition, mounting evidence supports the association between severe asthma and the IL-6 pathway.[26,102,103] Thus, a limitation of our study was the inability to test the effect of tocilizumab in patients with more severe asthma. Results from the PBS study demonstrated that, in individuals who were likely to suffer from asthma as well as rheumatoid arthritis, regular treatment with tocilizumab was not associated with a significant change in the frequency of ICS or salbutamol prescriptions. This suggests that regular tocilizumab treatment does not significantly attenuate or exacerbate asthma symptoms. In this analysis, cases likely included patients with more severe asthma. However, since the analysis was exclusively based on medical prescription data, we had no means of confirming our definitions of cases and controls, and the patterns of prescriptions analysed may not be an exact representation of medication use.

Future directions: Results from our clinical trial can be used to help design future trials with more refined hypotheses. For example, these could focus on drugs that specifically block IL-6 trans-signalling (e.g. olamkicept), and/or specifically address the possibility that anti-IL-6R therapy

is only effective in patients with rs4129267:TT genotype and mixed-granulocytic sputum subtype after challenge.

Overall conclusion

Collectively, the studies described in this thesis have (1) increased our understanding of how IL-6 signalling may contribute to asthma pathophysiology; and (2) provided new insights that can inform the design of future clinical studies of anti-IL-6R therapies in asthma.

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Appendix

APPENDIX

Supplementary Tables

Supplementary Table 3.1. Sputum inflammatory subtypes observed before (-24 h) and after (7 h and 24 h) allergen inhalation challenge.

N/A, no sputum sample available.

Participant	-24 h	7 h	24 h	Allergen
1	Eosinophilic	Eosinophilic	Eosinophilic	Cat
2	Eosinophilic	Eosinophilic	Eosinophilic	Cat
3	Eosinophilic	Eosinophilic	Eosinophilic	Cat
4	Eosinophilic	Eosinophilic	Eosinophilic	Cat
5	Eosinophilic	Eosinophilic	Eosinophilic	Cat
6	Eosinophilic	Eosinophilic	Eosinophilic	Cat
7	Eosinophilic	Eosinophilic	Eosinophilic	Cat
8	Eosinophilic	Eosinophilic	Eosinophilic	Cat
9	Eosinophilic	Eosinophilic	Eosinophilic	Cat
10	Eosinophilic	Eosinophilic	Eosinophilic	Cat
11	Eosinophilic	Eosinophilic	Eosinophilic	Cat
12	Eosinophilic	Eosinophilic	Eosinophilic	Cat
13	Eosinophilic	Eosinophilic	Eosinophilic	Cat
14	Eosinophilic	Eosinophilic	Eosinophilic	Cat
15	Eosinophilic	Eosinophilic	Eosinophilic	Cat
16	Eosinophilic	Eosinophilic	Eosinophilic	Grass
17	Eosinophilic	Eosinophilic	Eosinophilic	Grass
18	Eosinophilic	Eosinophilic	Eosinophilic	Grass
19	Eosinophilic	Eosinophilic	Eosinophilic	Grass
20	Eosinophilic	Eosinophilic	Eosinophilic	HDMDF
21	Eosinophilic	Eosinophilic	Eosinophilic	HDMDF
22	Eosinophilic	Eosinophilic	Eosinophilic	HDMDF
23	Eosinophilic	Eosinophilic	Eosinophilic	HDMDP
24	Eosinophilic	Eosinophilic	Eosinophilic	HDMDP
25	Eosinophilic	Eosinophilic	Eosinophilic	HDMDP
26	Eosinophilic	Eosinophilic	Eosinophilic	HDMDP
27	Eosinophilic	Eosinophilic	Eosinophilic	HDMDP
28	Eosinophilic	Eosinophilic	Eosinophilic	HDMDP
29	Eosinophilic	Eosinophilic	Eosinophilic	HDMDP
30	Eosinophilic	Eosinophilic	Eosinophilic	Ragweed
31	Eosinophilic	Eosinophilic	Eosinophilic	Ragweed
32	Eosinophilic	Eosinophilic	Mixed granulocytic	Grass
33	Eosinophilic	Eosinophilic	Mixed granulocytic	HDMDP
34	Eosinophilic	Eosinophilic	Paucigranulocytic	HDMDF
35	Eosinophilic	Mixed granulocytic	Eosinophilic	Cat
36	Eosinophilic	Mixed granulocytic	Eosinophilic	Cat
37	Eosinophilic	Mixed granulocytic	Eosinophilic	Cat
38	Eosinophilic	Mixed granulocytic	Eosinophilic	Cat
39	Eosinophilic	Mixed granulocytic	Eosinophilic	Grass

40	Eosinophilic	Mixed granulocytic	Eosinophilic	HDMDF
41	Eosinophilic	Mixed granulocytic	Eosinophilic	HDMDP
42	Eosinophilic	Mixed granulocytic	Eosinophilic	HDMDP
43	Eosinophilic	Mixed granulocytic	Eosinophilic	HDMDP
44	Eosinophilic	Mixed granulocytic	Eosinophilic	HDMDP
45	Eosinophilic	Mixed granulocytic	Eosinophilic	HDMDP
46	Eosinophilic	Mixed granulocytic	Mixed granulocytic	Cat
47	Eosinophilic	Mixed granulocytic	Mixed granulocytic	HDMDF
48	Eosinophilic	Mixed granulocytic	Mixed granulocytic	HDMDP
49	Eosinophilic	Mixed granulocytic	Mixed granulocytic	HDMDP
50	Eosinophilic	N/A	Eosinophilic	Grass
51	Eosinophilic	N/A	Eosinophilic	HDMDP
52	Mixed granulocytic	Eosinophilic	Eosinophilic	Cat
53	Mixed granulocytic	Eosinophilic	Eosinophilic	HDMDP
54	Mixed granulocytic	Eosinophilic	Eosinophilic	HDMDP
55	Mixed granulocytic	Eosinophilic	Mixed granulocytic	Cat
56	Mixed granulocytic	Eosinophilic	Mixed granulocytic	Cat
57	Mixed granulocytic	Eosinophilic	Mixed granulocytic	HDMDP
58	Mixed granulocytic	Mixed granulocytic	Mixed granulocytic	Cat
59	Mixed granulocytic	Mixed granulocytic	Mixed granulocytic	Grass
60	Mixed granulocytic	Mixed granulocytic	Mixed granulocytic	Ragweed
61	Mixed granulocytic	Mixed granulocytic	Mixed granulocytic	Ragweed
62	Mixed granulocytic	N/A	Eosinophilic	Grass
63	Neutrophilic	Eosinophilic	Eosinophilic	HDMDF
64	Neutrophilic	Eosinophilic	Eosinophilic	Ragweed
65	Neutrophilic	Eosinophilic	Paucigranulocytic	HDMDF
66	Neutrophilic	Mixed granulocytic	Eosinophilic	HDMDF
67	Neutrophilic	Mixed granulocytic	Eosinophilic	HDMDP
68	Neutrophilic	Mixed granulocytic	Mixed granulocytic	Cat
69	Neutrophilic	Mixed granulocytic	Mixed granulocytic	Grass
70	Neutrophilic	Mixed granulocytic	Mixed granulocytic	HDMDP
71	Neutrophilic	Mixed granulocytic	Mixed granulocytic	HDMDP
72	Neutrophilic	Mixed granulocytic	N/A	Ragweed
73	Neutrophilic	Neutrophilic	Neutrophilic	Grass
74	Paucigranulocytic	Eosinophilic	Eosinophilic	Cat
75	Paucigranulocytic	Eosinophilic	Eosinophilic	Cat
76	Paucigranulocytic	Eosinophilic	Eosinophilic	Cat
77	Paucigranulocytic	Eosinophilic	Eosinophilic	Cat
78	Paucigranulocytic	Eosinophilic	Eosinophilic	Cat
79	Paucigranulocytic	Eosinophilic	Eosinophilic	Cat
80	Paucigranulocytic	Eosinophilic	Eosinophilic	Grass
81	Paucigranulocytic	Eosinophilic	Eosinophilic	Grass
82	Paucigranulocytic	Eosinophilic	Eosinophilic	HDMDP
83	Paucigranulocytic	Eosinophilic	Eosinophilic	HDMDP
84	Paucigranulocytic	Eosinophilic	Eosinophilic	HDMDP
85	Paucigranulocytic	Eosinophilic	Eosinophilic	HDMDP
86	Paucigranulocytic	Eosinophilic	Eosinophilic	HDMDP
87	Paucigranulocytic	Eosinophilic	Eosinophilic	HDMDP
88	Paucigranulocytic	Eosinophilic	Eosinophilic	HDMDP

89	Paucigranulocytic	Eosinophilic	Eosinophilic	Ragweed
90	Paucigranulocytic	Eosinophilic	Eosinophilic	Ragweed
91	Paucigranulocytic	Eosinophilic	Eosinophilic	Ragweed
92	Paucigranulocytic	Eosinophilic	Eosinophilic	Ragweed
93	Paucigranulocytic	Eosinophilic	Mixed granulocytic	Grass
94	Paucigranulocytic	Eosinophilic	N/A	Ragweed
95	Paucigranulocytic	Mixed granulocytic	Eosinophilic	Cat
96	Paucigranulocytic	Mixed granulocytic	Eosinophilic	Cat
97	Paucigranulocytic	Mixed granulocytic	Eosinophilic	Cat
98	Paucigranulocytic	Mixed granulocytic	Eosinophilic	Grass
99	Paucigranulocytic	Mixed granulocytic	Eosinophilic	Grass
100	Paucigranulocytic	Mixed granulocytic	Eosinophilic	Grass
101	Paucigranulocytic	Mixed granulocytic	Eosinophilic	Grass
102	Paucigranulocytic	Mixed granulocytic	Eosinophilic	Grass
103	Paucigranulocytic	Mixed granulocytic	Eosinophilic	HDMDF
104	Paucigranulocytic	Mixed granulocytic	Eosinophilic	HDMDP
105	Paucigranulocytic	Mixed granulocytic	Eosinophilic	HDMDP
106	Paucigranulocytic	Mixed granulocytic	Eosinophilic	HDMDP
107	Paucigranulocytic	Mixed granulocytic	Eosinophilic	HDMDP
108	Paucigranulocytic	Mixed granulocytic	Eosinophilic	Ragweed
109	Paucigranulocytic	Mixed granulocytic	Mixed granulocytic	Grass
110	Paucigranulocytic	Mixed granulocytic	Mixed granulocytic	HDMDP
111	Paucigranulocytic	Mixed granulocytic	Neutrophilic	Ragweed
112	Paucigranulocytic	Mixed granulocytic	Paucigranulocytic	HDMDP
113	Paucigranulocytic	Mixed granulocytic	N/A	Cat
114	Paucigranulocytic	Paucigranulocytic	Eosinophilic	HDMDP
115	Paucigranulocytic	Paucigranulocytic	Eosinophilic	Ragweed
116	Paucigranulocytic	Paucigranulocytic	Paucigranulocytic	HDMDP
117	Paucigranulocytic	Paucigranulocytic	Paucigranulocytic	Ragweed
118	Paucigranulocytic	N/A	Mixed granulocytic	Cat
119	N/A	Eosinophilic	Eosinophilic	Cat
120	N/A	Eosinophilic	Eosinophilic	HDMDF
121	N/A	Eosinophilic	Eosinophilic	HDMDP
122	N/A	Eosinophilic	N/A	HDMDP
123	N/A	Mixed granulocytic	Mixed granulocytic	Grass
124	N/A	Mixed granulocytic	Mixed granulocytic	HDMDF
125	N/A	Paucigranulocytic	Eosinophilic	Cat
126	N/A	Paucigranulocytic	Eosinophilic	Cat
127	N/A	Paucigranulocytic	Eosinophilic	Ragweed
128	N/A	N/A	Eosinophilic	Grass
129	N/A	N/A	N/A	HDMDP

Supplementary Table 4.1. 2,203 genes co-expressed with *IL6R* in the GEUVADIS and DMAP studies.

Abbreviations: Chr, chromosome; Cor, correlation.

		Geuvadis		DMAP	
Gene	Chr	Cor	*P*-value	Cor	*P*-value
FYN	6	-0.236	3.95E-06	0.413	4.14E-10
CD180	5	0.234	4.70E-06	0.181	0.0083
ATP8B2	1	0.230	6.89E-06	0.027	0.6949
NEURL3	2	0.230	7.52E-06	NA	NA
CCL22	16	0.221	1.59E-05	0.056	0.4144
CASK-AS1	X	0.219	1.95E-05	NA	NA
SYBU	8	0.213	3.27E-05	NA	NA
TRIM47	17	0.211	3.92E-05	NA	NA
FAM104A	17	-0.207	5.49E-05	NA	NA
CACNA1A	19	0.206	6.11E-05	-0.313	3.62E-06
NCR2	6	-0.205	6.90E-05	NA	NA
TBKBP1	17	-0.203	7.71E-05	NA	NA
IL18R1	2	-0.202	8.25E-05	-0.008	0.9073
RNF213	17	0.199	0.0001	NA	NA
KYNU	2	0.197	0.0001	0.262	0.0001
KLF10	8	0.196	0.0001	0.023	0.7387
RARRES3	11	0.193	0.0002	0.175	0.0110
FAM65B	6	0.193	0.0002	NA	NA
B3GNT2	2	-0.193	0.0002	-0.335	6.45E-07
ITM2C	2	0.193	0.0002	-0.179	0.0092
RGS2	1	-0.191	0.0002	0.237	0.0005
BCL9L	11	0.189	0.0002	NA	NA
DPYSL2	8	0.188	0.0003	0.186	0.0066
NCK2	2	0.188	0.0003	0.195	0.0045
SPSB1	1	0.188	0.0003	NA	NA
CISH	3	0.187	0.0003	0.318	2.51E-06
ATPIF1	1	-0.185	0.0003	-0.042	0.5476
CUEDC1	17	0.185	0.0003	0.084	0.2216
STX5	11	-0.184	0.0004	0.097	0.1605
RIN3	14	-0.183	0.0004	0.357	9.63E-08
EEF1A1P38	16	0.182	0.0004	NA	NA
GFPT1	2	-0.182	0.0004	0.114	0.0984
ARHGAP11A	15	-0.181	0.0004	-0.123	0.0748
RPL3P9	8	0.180	0.0005	NA	NA
MOXD1	6	-0.180	0.0005	-0.174	0.0115
KLHL29	2	0.180	0.0005	NA	NA
PLCL2	3	-0.180	0.0005	0.068	0.3232
ZNF697	1	0.179	0.0005	NA	NA

AHNAK	11	0.179	0.0005	0.238	0.0005
CBLB	3	-0.178	0.0006	-0.010	0.8903
HIPK2	7	0.178	0.0006	-0.063	0.3610
ZFP91	11	-0.177	0.0006	NA	NA
CPNE5	6	0.177	0.0006	NA	NA
ITPR3	6	0.176	0.0006	-0.031	0.6506
KLF2	19	0.175	0.0007	0.275	5.27E-05
EPS8	12	0.175	0.0007	0.429	7.80E-11
PTGIR	19	0.175	0.0007	NA	NA
FAM8A1	6	0.175	0.0007	0.093	0.1802
SLC25A5P5	7	0.173	0.0008	NA	NA
MAP3K5	6	-0.172	0.0009	0.261	0.0001
VDR	12	0.171	0.0009	0.391	4.15E-09
TBX21	17	-0.171	0.0009	-0.175	0.0107
ZNF329	19	-0.171	0.0009	-0.080	0.2486
BICD1	12	0.171	0.0009	-0.310	4.39E-06
CORO1B	11	0.171	0.0009	0.469	6.50E-13
CEACAM1	19	0.171	0.0009	0.185	0.0071
ZNF84	12	-0.170	0.0010	-0.053	0.4419
LIMK1	7	-0.170	0.0010	0.040	0.5633
COLGALT2	1	0.169	0.0010	NA	NA
HNF4G	8	0.169	0.0010	NA	NA
SLC25A37	8	0.169	0.0010	-0.090	0.1945
ZNF570	19	-0.169	0.0010	NA	NA
LRRC32	11	0.169	0.0010	NA	NA
SLC25A32	8	-0.169	0.0011	0.002	0.9769
MPZL2	11	0.169	0.0011	NA	NA
MSN	X	0.169	0.0011	0.180	0.0087
AQR	15	0.168	0.0011	0.209	0.0023
UBAC2	13	-0.168	0.0012	NA	NA
SAMSN1	21	-0.167	0.0012	0.333	7.46E-07
AP3D1	19	-0.167	0.0012	0.184	0.0074
PSIP1	9	-0.167	0.0012	-0.012	0.8620
RPL5P35	22	0.167	0.0012	NA	NA
MFHAS1	8	0.166	0.0013	-0.024	0.7248
EEF1A1P14	1	0.166	0.0013	NA	NA
PRKCH	14	-0.165	0.0014	0.155	0.0243
APBB1IP	10	0.165	0.0014	0.288	2.19E-05
RPL7AP31	4	0.165	0.0014	NA	NA
SEMA4B	15	0.165	0.0014	NA	NA
TMCC2	1	0.164	0.0015	-0.171	0.0128
MYL12AP1	8	0.164	0.0015	NA	NA
ARID3A	19	0.162	0.0017	0.091	0.1877
FECH	18	-0.162	0.0018	-0.188	0.0063
FAM46C	1	0.161	0.0018	-0.301	8.60E-06

TBC1D23	3	-0.161	0.0018	NA	NA
ANKRD33B	5	0.161	0.0018	NA	NA
CTSZ	20	0.161	0.0019	0.210	0.0021
UFL1	6	-0.161	0.0019	NA	NA
TOMM70A	3	-0.160	0.0019	0.074	0.2816
PLEKHM2	1	-0.160	0.0019	NA	NA
GPR18	13	-0.160	0.0019	0.189	0.0058
G0S2	1	0.160	0.0020	0.302	8.23E-06
MBOAT2	2	0.159	0.0021	-0.254	0.0002
RAB37	17	-0.159	0.0021	NA	NA
BCL7A	12	0.158	0.0022	-0.360	7.78E-08
CD1C	1	0.158	0.0023	0.300	9.24E-06
CLINT1	5	0.158	0.0023	0.030	0.6605
TLE1	9	0.158	0.0023	-0.305	6.50E-06
CAMK2D	4	-0.156	0.0025	-0.001	0.9882
ABCA5	17	-0.156	0.0025	-0.020	0.7723
SORBS3	8	0.156	0.0025	NA	NA
SEMA6A	5	0.156	0.0025	-0.092	0.1835
ECHS1	10	-0.156	0.0026	0.070	0.3123
FEZ1	11	-0.155	0.0026	-0.018	0.7953
ZFP41	8	0.155	0.0026	NA	NA
SMIM3	5	-0.155	0.0027	NA	NA
ERICH5	8	0.155	0.0027	NA	NA
RPL17P50	10	0.155	0.0027	NA	NA
FCHO1	19	0.154	0.0028	NA	NA
GOLGA6L5P	15	-0.154	0.0028	NA	NA
SYCP2	20	-0.154	0.0028	NA	NA
RPL7P26	6	0.154	0.0029	NA	NA
PABPC1P1	4	0.154	0.0029	NA	NA
BIRC3	11	0.154	0.0029	0.286	2.46E-05
NAPSB	19	0.153	0.0030	NA	NA
C3orf20	3	-0.153	0.0030	NA	NA
RILPL2	12	-0.153	0.0031	NA	NA
RGS14	5	0.152	0.0032	0.340	4.32E-07
F13A1	6	-0.152	0.0032	0.295	1.34E-05
DNAH17	17	-0.152	0.0032	0.357	1.01E-07
GHITM	10	-0.151	0.0035	0.012	0.8673
GNGT2	17	-0.150	0.0036	0.212	0.0020
CLSTN1	1	-0.150	0.0036	0.175	0.0109
LAMB3	1	0.150	0.0037	NA	NA
FRMD3	9	0.150	0.0038	NA	NA
TM6SF1	15	-0.149	0.0038	0.292	1.59E-05
SMIM10	X	0.149	0.0040	NA	NA
MAPK6PS3	13	-0.149	0.0040	NA	NA
GLUD2	X	-0.149	0.0040	0.273	5.75E-05

EFNB2	13	0.149	0.0040	NA	NA
MYO1D	17	0.149	0.0040	NA	NA
FAM129C	19	0.148	0.0041	NA	NA
CDC42EP4	17	0.148	0.0042	-0.015	0.8234
HCG27	6	0.148	0.0042	NA	NA
NUDT8	11	-0.147	0.0043	NA	NA
GBF1	10	-0.147	0.0043	-0.030	0.6657
SLC14A1	18	-0.147	0.0044	-0.215	0.0017
MYO5C	15	0.147	0.0045	-0.210	0.0022
RBMS3	3	0.147	0.0045	NA	NA
NIPAL1	4	0.147	0.0046	NA	NA
CAPG	2	0.146	0.0046	0.274	5.35E-05
POU5F1B	8	0.146	0.0046	NA	NA
MSNP1	5	0.146	0.0046	NA	NA
PARM1	4	0.146	0.0047	NA	NA
ALOX12-AS1	17	-0.146	0.0047	NA	NA
DSTYK	1	0.146	0.0048	NA	NA
ABI3	17	-0.146	0.0048	NA	NA
FZR1	19	-0.146	0.0048	-0.062	0.3693
ATL1	14	0.145	0.0049	NA	NA
DNMBP-AS1	10	-0.145	0.0050	NA	NA
SLC25A19	17	0.145	0.0050	NA	NA
YRDC	1	-0.145	0.0051	-0.060	0.3880
NPIPP1	16	-0.144	0.0052	NA	NA
CDC20P1	9	-0.144	0.0052	NA	NA
SESN3	11	0.144	0.0052	NA	NA
SIRPB1	20	0.144	0.0053	0.570	0
ARHGAP31	3	0.144	0.0053	NA	NA
RPL3P1	21	0.144	0.0053	NA	NA
DPF3	14	-0.144	0.0053	NA	NA
HIST1H3PS1	6	0.144	0.0053	NA	NA
GLDC	9	0.144	0.0053	-0.084	0.2221
PSD3	8	0.144	0.0053	-0.207	0.0025
SOAT1	1	-0.144	0.0053	0.463	1.36E-12
TPMT	6	0.144	0.0055	0.240	0.0004
MGAT4A	2	0.143	0.0055	0.308	5.24E-06
IL18RAP	2	-0.143	0.0057	-0.143	0.0373
CREB3	9	-0.142	0.0059	NA	NA
NRD1	1	-0.142	0.0059	0.100	0.1470
HLTF	3	-0.142	0.0059	-0.215	0.0017
GLTPP1	11	-0.142	0.0060	NA	NA
NR3C1	5	0.142	0.0061	0.220	0.0013
TCL6	14	0.141	0.0062	-0.176	0.0106
RASGRP2	11	0.141	0.0064	0.131	0.0584
ZNF425	7	0.141	0.0064	NA	NA

CXCR5	11	0.141	0.0065	NA	NA
TBC1D17	19	-0.141	0.0065	NA	NA
RAP1GDS1	4	-0.141	0.0065	0.158	0.0218
RBBP4P2	3	0.140	0.0067	NA	NA
HMGB3P6	1	-0.140	0.0067	NA	NA
ITM2A	X	-0.140	0.0067	-0.130	0.0591
SIGIRR	11	0.140	0.0069	0.236	0.0006
MALT1	18	-0.140	0.0069	0.367	4.14E-08
RELA	11	-0.140	0.0069	0.189	0.0059
KL	13	0.140	0.0070	0.116	0.0941
SUPT5H	19	-0.139	0.0070	0.165	0.0161
GCAT	22	-0.139	0.0071	NA	NA
EFCAB7	1	0.139	0.0072	NA	NA
ZNF540	19	-0.139	0.0072	NA	NA
PPP3CA	4	-0.139	0.0073	-0.103	0.1365
YIF1B	19	-0.139	0.0073	NA	NA
IER5L	9	0.139	0.0073	NA	NA
TNS1	2	0.139	0.0073	-0.200	0.0035
C9orf142	9	0.139	0.0074	NA	NA
CALHM1	10	0.138	0.0074	NA	NA
HCAR1	12	0.138	0.0074	NA	NA
SNHG10	14	0.138	0.0074	NA	NA
USP46	4	0.138	0.0074	0.089	0.1993
HSPD1P2	8	0.138	0.0075	NA	NA
ETV4	17	-0.138	0.0076	NA	NA
BORA	13	-0.138	0.0076	NA	NA
ZBED3-AS1	5	0.138	0.0076	NA	NA
FKBP8	19	-0.138	0.0076	-0.145	0.0355
LRRC42	1	-0.138	0.0077	NA	NA
FAM207BP	13	0.138	0.0078	NA	NA
FAM174B	15	-0.137	0.0079	NA	NA
PFKFB1	X	-0.137	0.0079	NA	NA
AQP3	9	0.137	0.0079	0.184	0.0074
RPL18AP16	X	0.137	0.0079	NA	NA
RPL7AP73	13	0.137	0.0081	NA	NA
SUPT3H	6	0.137	0.0082	0.083	0.2289
TMC8	17	-0.137	0.0082	NA	NA
NDFIP1	5	-0.137	0.0083	0.439	2.40E-11
TET2	4	-0.136	0.0083	NA	NA
JUN	1	0.136	0.0085	-0.075	0.2800
TMEM51	1	-0.136	0.0085	0.207	0.0025
PPM1M	3	0.136	0.0085	NA	NA
SFR1P1	X	0.136	0.0085	NA	NA
DSC2	18	-0.136	0.0085	0.388	5.45E-09
EEF1A1P30	X	0.136	0.0085	NA	NA

SS18L1	20	-0.136	0.0085	0.238	0.0005
NR4A3	9	0.136	0.0086	-0.032	0.6488
NDUFS1	2	-0.136	0.0086	0.169	0.0142
CCDC26	8	-0.136	0.0086	NA	NA
TMEM147	19	0.136	0.0086	0.121	0.0804
ACTG1P2	Y	0.136	0.0086	NA	NA
NFIB	9	0.136	0.0087	-0.185	0.0069
WDFY1	2	-0.136	0.0087	NA	NA
C9orf156	9	0.136	0.0087	0.267	8.77E-05
KLF3	4	-0.136	0.0087	0.117	0.0903
LTA	6	0.136	0.0088	0.039	0.5715
TRAFD1	12	0.136	0.0088	0.336	5.63E-07
ARF4	3	-0.136	0.0088	-0.010	0.8881
CDK9	9	-0.135	0.0088	NA	NA
KLHL36	16	-0.135	0.0088	NA	NA
SLFN11	17	0.135	0.0088	NA	NA
C21orf91	21	-0.135	0.0088	0.017	0.8051
PDZD2	5	0.135	0.0088	-0.240	0.0004
CKB	14	0.135	0.0088	NA	NA
TBC1D16	17	-0.135	0.0090	-0.140	0.0422
RPL36AP13	2	0.135	0.0090	NA	NA
NCR3LG1	11	-0.135	0.0090	NA	NA
VAMP5	2	-0.135	0.0090	NA	NA
MED11	17	0.135	0.0090	NA	NA
SGMS1-AS1	10	-0.135	0.0091	NA	NA
ASB2	14	0.135	0.0091	NA	NA
ATP1B2	17	0.135	0.0091	-0.179	0.0092
CACYBPP1	10	0.135	0.0092	NA	NA
FOXA3	19	0.135	0.0092	NA	NA
PIK3R5	17	0.134	0.0093	0.235	0.0006
YBX1P10	9	-0.134	0.0094	NA	NA
PA2G4P1	X	0.134	0.0094	NA	NA
MAP4	3	-0.134	0.0094	0.104	0.1309
H3F3AP5	X	0.134	0.0094	NA	NA
EEF1A1P17	12	0.134	0.0096	NA	NA
DNASE1L3	3	-0.134	0.0096	-0.038	0.5826
DTX3L	3	0.134	0.0096	NA	NA
TRGV2	7	0.134	0.0097	NA	NA
HMSD	18	0.134	0.0098	NA	NA
ATAD2B	2	0.134	0.0098	0.146	0.0340
NUAK2	1	0.134	0.0098	NA	NA
SYNGR3	16	-0.134	0.0098	NA	NA
SMAGP	12	0.134	0.0098	NA	NA
MCL1	1	0.133	0.0099	0.478	2.03E-13
FCRL3	1	-0.133	0.0099	NA	NA

VEZF1	17	0.133	0.0099	0.194	0.0047
VDAC3	8	-0.133	0.0099	0.025	0.7143
MORF4L1P1	1	-0.133	0.0100	NA	NA
INPP5F	10	0.133	0.0101	0.101	0.1423
WDR33	2	-0.133	0.0101	0.142	0.0393
ORC6	16	-0.133	0.0101	NA	NA
EPB41L5	2	0.133	0.0101	-0.072	0.2965
HIVEP3	1	0.133	0.0101	-0.037	0.5889
ERCC6L	X	-0.133	0.0102	NA	NA
B4GALT2	1	-0.133	0.0102	-0.079	0.2513
TBC1D9	4	0.133	0.0102	0.149	0.0305
ANKRD13B	17	0.133	0.0102	NA	NA
PTBP3	9	-0.133	0.0103	NA	NA
LINC01358	1	0.133	0.0104	NA	NA
ZFR	5	-0.132	0.0105	0.059	0.3951
ZMYM5	13	-0.132	0.0105	0.017	0.8032
KLF7	2	0.132	0.0106	0.313	3.65E-06
BTF3P8	X	0.132	0.0107	NA	NA
PRDX1P1	9	0.132	0.0107	NA	NA
CDC20	1	-0.132	0.0107	-0.308	5.13E-06
HMX3	10	0.132	0.0107	NA	NA
STT3B	3	-0.132	0.0110	NA	NA
POU3F1	1	0.132	0.0110	-0.097	0.1591
ENSA	1	0.131	0.0110	-0.211	0.0020
PPP6R1	19	-0.131	0.0111	NA	NA
DENND1C	19	-0.131	0.0111	0.226	0.0010
CHM	X	0.131	0.0111	NA	NA
PQLC2	1	0.131	0.0111	NA	NA
PPP1R14BP3	4	-0.131	0.0112	NA	NA
ESR1	6	0.131	0.0114	0.151	0.0282
HMGB3	X	-0.131	0.0114	-0.226	0.0009
MYO1F	19	0.131	0.0116	0.297	1.13E-05
LHFPL2	5	0.130	0.0117	-0.203	0.0031
TMEM140	7	0.130	0.0117	NA	NA
KLHDC2	14	-0.130	0.0117	-0.049	0.4814
R3HDM4	19	0.130	0.0118	NA	NA
SHE	1	0.130	0.0118	NA	NA
ACBD4	17	-0.130	0.0119	NA	NA
SERPINH1P1	9	-0.130	0.0119	NA	NA
RPS3AP25	7	0.130	0.0119	NA	NA
MTMR9LP	1	0.130	0.0121	NA	NA
PURB	7	-0.130	0.0121	NA	NA
SNIP1	1	0.130	0.0121	0.056	0.4202
ARHGEF2	1	0.130	0.0122	0.069	0.3195
IQCH	15	-0.130	0.0122	NA	NA

SLC22A23	6	0.130	0.0122	NA	NA
COMMD8	4	-0.130	0.0123	0.385	7.08E-09
ZBP1	20	0.129	0.0123	0.097	0.1613
PYCARD	16	0.129	0.0123	0.313	3.66E-06
PLA1A	3	0.129	0.0124	0.092	0.1816
CFAP57	1	0.129	0.0125	NA	NA
TSKU	11	0.129	0.0125	NA	NA
LAMA3	18	0.129	0.0125	NA	NA
NDUFAF4	6	-0.129	0.0126	NA	NA
GRB2	17	0.129	0.0126	0.208	0.0024
BIRC5	17	-0.129	0.0126	-0.322	1.82E-06
OSBPL3	7	0.129	0.0126	0.017	0.8029
TMEM173	5	0.129	0.0127	NA	NA
SEMA3F-AS1	3	-0.129	0.0127	NA	NA
MICAL1	6	-0.129	0.0127	0.330	9.50E-07
SLC20A1	2	0.129	0.0127	0.107	0.1213
FAM78B	1	0.129	0.0127	NA	NA
RPL26P37	Y	0.129	0.0128	NA	NA
DCTN3	9	-0.129	0.0128	0.027	0.6916
MRGBP	20	-0.129	0.0128	NA	NA
SH3PXD2A	10	0.129	0.0128	-0.011	0.8715
MED12L	3	0.129	0.0129	NA	NA
MOB3B	9	0.129	0.0129	NA	NA
GAPDHP70	11	0.129	0.0129	NA	NA
TMED9	5	-0.128	0.0130	0.157	0.0226
CTDSP2	12	0.128	0.0130	0.165	0.0166
NCOA1	2	-0.128	0.0131	0.193	0.0050
TTYH3	7	-0.128	0.0131	NA	NA
TMEM80	11	-0.128	0.0132	0.158	0.0218
UHMK1	1	-0.128	0.0132	NA	NA
ATP6V1G1	9	0.128	0.0132	0.137	0.0474
MAD2L1	4	-0.128	0.0132	-0.244	0.0004
PKIG	20	-0.128	0.0133	-0.468	7.26E-13
GAS2	11	0.128	0.0133	-0.007	0.9169
KIAA0232	4	-0.128	0.0133	0.235	0.0006
GALNT10	5	-0.128	0.0133	0.018	0.7937
WNT5B	12	0.128	0.0135	NA	NA
FOXD2-AS1	1	0.128	0.0135	NA	NA
HIST2H2BF	1	0.128	0.0136	NA	NA
GRAMD4	22	0.128	0.0136	NA	NA
RASAL2	1	0.128	0.0136	-0.083	0.2301
CYBB	X	0.128	0.0136	0.296	1.26E-05
HECW2	2	-0.128	0.0136	NA	NA
IFNGR2	21	0.128	0.0137	0.329	1.04E-06
HMX2	10	0.128	0.0137	NA	NA

MED28	4	-0.128	0.0137	-0.094	0.1725
GLT1D1	12	-0.127	0.0138	NA	NA
GABARAP	17	-0.127	0.0138	0.196	0.0043
DAB2IP	9	-0.127	0.0139	NA	NA
YARS	1	-0.127	0.0139	-0.105	0.1285
RPL23AP12	21	0.127	0.0139	NA	NA
HABP4	9	0.127	0.0140	0.188	0.0063
DUX4L50	9	0.127	0.0140	NA	NA
METTL18	1	-0.127	0.0140	NA	NA
DIS3	13	-0.127	0.0140	NA	NA
RPL5P11	4	0.127	0.0141	NA	NA
BMP6	6	0.127	0.0141	-0.055	0.4305
ZBTB12	6	-0.127	0.0141	NA	NA
SLC4A1AP	2	-0.127	0.0141	0.129	0.0615
IFI27L1	14	-0.127	0.0142	NA	NA
EEF1A1P10	7	0.127	0.0143	NA	NA
PTEN	10	-0.127	0.0143	0.196	0.0043
ORAI3	16	-0.127	0.0143	NA	NA
MTATP8P1	1	-0.127	0.0144	NA	NA
CNN3	1	0.127	0.0144	-0.235	0.0006
CCL17	16	0.126	0.0145	NA	NA
LSP1P3	5	0.126	0.0146	NA	NA
SGOL1	3	-0.126	0.0146	NA	NA
RASAL3	19	-0.126	0.0147	NA	NA
BSCL2	11	-0.126	0.0147	NA	NA
CASKIN1	16	0.126	0.0148	NA	NA
RPL23AP38	3	0.126	0.0148	NA	NA
SNAI1	20	0.126	0.0148	NA	NA
SERBP1P1	X	-0.126	0.0149	NA	NA
OGDHL	10	-0.126	0.0149	NA	NA
DQX1	2	0.126	0.0149	NA	NA
NUDT19	19	-0.126	0.0150	NA	NA
TNFRSF11B	8	0.126	0.0151	NA	NA
SLCO3A1	15	0.126	0.0152	0.597	0
CNKSR1	1	0.126	0.0152	NA	NA
TANK	2	0.126	0.0153	-0.005	0.9389
PPAP2B	1	0.125	0.0153	-0.211	0.0021
SNHG14	15	-0.125	0.0153	NA	NA
LRBA	4	-0.125	0.0153	-0.191	0.0053
NSUN3	3	-0.125	0.0154	-0.060	0.3883
SFXN4	10	-0.125	0.0154	NA	NA
NPM1P33	2	0.125	0.0155	NA	NA
TSG101	11	0.125	0.0155	-0.014	0.8361
PPP4R2	3	-0.125	0.0155	-0.065	0.3495
LGALS9C	17	-0.125	0.0155	NA	NA

PALLD	4	0.125	0.0156	0.095	0.1706
LAMP5	20	-0.125	0.0157	NA	NA
ZNF706	8	-0.125	0.0157	0.143	0.0377
ABHD6	3	-0.125	0.0157	0.019	0.7816
RPS12P23	13	0.125	0.0157	NA	NA
LMNTD2	11	-0.125	0.0157	NA	NA
YIPF6	X	0.125	0.0158	-0.094	0.1754
ADM	11	0.125	0.0159	0.209	0.0023
EPHA1-AS1	7	0.125	0.0159	NA	NA
RPL32	3	0.125	0.0160	0.065	0.3501
KIF4B	5	-0.125	0.0160	NA	NA
ACTG1P14	9	0.125	0.0160	NA	NA
ATP5J2	7	0.125	0.0160	0.026	0.7021
GPR183	13	-0.125	0.0160	NA	NA
PITPNC1	17	0.124	0.0162	0.271	6.77E-05
RPL36AP26	7	0.124	0.0162	NA	NA
CPS1	2	0.124	0.0162	-0.135	0.0495
RTKN	2	0.124	0.0163	NA	NA
CEACAM21	19	-0.124	0.0163	NA	NA
NOC4L	12	0.124	0.0164	-0.032	0.6481
DLL4	15	0.124	0.0165	NA	NA
NFAM1	22	-0.124	0.0165	NA	NA
IL12B	5	0.124	0.0166	NA	NA
BCL2A1	15	0.124	0.0167	0.366	4.50E-08
CXCR4	2	0.124	0.0167	0.239	0.0005
RAP1BP1	9	0.124	0.0167	NA	NA
VOPP1	7	0.124	0.0168	NA	NA
RPL7P48	17	-0.124	0.0168	NA	NA
PIAS2	18	-0.124	0.0170	-0.060	0.3886
ZMYM4	1	0.123	0.0170	0.106	0.1243
SH2D3C	9	-0.123	0.0171	NA	NA
RAB42	1	0.123	0.0171	NA	NA
LRRC8C	1	-0.123	0.0172	NA	NA
PSMB10	16	-0.123	0.0172	0.285	2.70E-05
TNFRSF8	1	0.123	0.0172	0.289	1.96E-05
LGALS14	19	-0.123	0.0172	NA	NA
FITM1	14	-0.123	0.0173	NA	NA
HTR2B	2	0.123	0.0174	0.118	0.0880
SMIM7	19	-0.123	0.0174	NA	NA
FAM222B	17	0.123	0.0174	NA	NA
SLC44A2	19	-0.123	0.0175	NA	NA
ZNF355P	21	0.123	0.0177	NA	NA
C2orf44	2	0.123	0.0177	-0.097	0.1608
GAPT	5	0.123	0.0177	NA	NA
NCKAP5	2	0.123	0.0178	NA	NA

HYOU1	11	-0.123	0.0178	-0.010	0.8886
KCNK5	6	-0.123	0.0178	-0.289	1.94E-05
TMC4	19	0.123	0.0178	NA	NA
TMX1	14	0.123	0.0179	NA	NA
WWC3	X	-0.123	0.0179	0.051	0.4621
ANXA2P2	9	0.122	0.0180	0.409	6.58E-10
PPP2R2A	8	-0.122	0.0180	0.150	0.0291
JDP2	14	0.122	0.0180	NA	NA
NLRP3	1	0.122	0.0181	0.484	8.37E-14
LCA5	6	0.122	0.0182	NA	NA
CENPJ	13	-0.122	0.0184	-0.331	8.84E-07
TCF19	6	0.122	0.0185	NA	NA
GYS1	19	-0.122	0.0186	0.196	0.0043
CD300C	17	-0.122	0.0186	0.189	0.0059
ANKRA2	5	-0.122	0.0188	0.139	0.0433
RBBP7	X	0.121	0.0189	-0.108	0.1177
SEC24B-AS1	4	-0.121	0.0189	NA	NA
ANKRD16	10	0.121	0.0190	NA	NA
YBX1P2	7	-0.121	0.0191	NA	NA
RNF144A	2	-0.121	0.0191	NA	NA
GPCPD1	20	-0.121	0.0191	NA	NA
GPR63	6	-0.121	0.0192	NA	NA
UBE2G1	17	0.121	0.0192	0.099	0.1505
SRSF10	1	0.121	0.0192	NA	NA
B4GALT1	9	-0.121	0.0192	-0.022	0.7495
GBP1	1	-0.121	0.0193	0.389	4.85E-09
RPS21P4	4	0.121	0.0193	NA	NA
RXRA	9	0.121	0.0194	0.151	0.0285
RPS17P2	5	0.121	0.0194	NA	NA
TXNL1	18	-0.121	0.0195	-0.130	0.0597
TRABD2A	2	0.121	0.0195	NA	NA
ARAP1-AS1	11	-0.121	0.0196	NA	NA
MAPK6	15	-0.121	0.0197	0.120	0.0822
SLC18A2	10	0.121	0.0197	NA	NA
SRP54	14	-0.121	0.0198	0.183	0.0076
MTCH2	11	-0.121	0.0198	0.049	0.4827
RBMXP4	4	-0.121	0.0198	NA	NA
ZSCAN30	18	-0.121	0.0199	NA	NA
SOWAHD	X	0.120	0.0200	NA	NA
HNRNPA1P33	10	0.120	0.0201	NA	NA
ABI1	10	0.120	0.0201	0.122	0.0761
ADD2	2	0.120	0.0202	-0.229	0.0008
UBR3	2	-0.120	0.0202	NA	NA
PRORSD1P	2	-0.120	0.0202	NA	NA
LIPH	3	-0.120	0.0203	NA	NA

MED10	5	-0.120	0.0203	NA	NA
C11orf24	11	-0.120	0.0204	-0.095	0.1696
ICK	6	0.120	0.0205	0.151	0.0285
STPG1	1	-0.120	0.0206	NA	NA
ANXA11	10	0.120	0.0206	0.346	2.44E-07
FNIP2	4	-0.120	0.0206	NA	NA
FAM207A	21	0.120	0.0208	NA	NA
BCAP29	7	-0.120	0.0209	-0.182	0.0081
ANKDD1A	15	0.120	0.0209	0.014	0.8412
RDH14	2	0.119	0.0210	-0.093	0.1766
DNAJC3	13	-0.119	0.0211	0.177	0.0099
PMAIP1	18	-0.119	0.0211	-0.237	0.0005
FCHO2	5	0.119	0.0211	NA	NA
ADSL	22	-0.119	0.0211	0.200	0.0035
IGLV1-47	22	0.119	0.0212	NA	NA
SAPCD2	9	0.119	0.0212	NA	NA
MAPK1	22	0.119	0.0214	0.033	0.6381
NPM1P35	11	0.119	0.0214	NA	NA
TNFRSF1B	1	-0.119	0.0214	0.437	3.14E-11
GPR55	2	0.119	0.0215	NA	NA
PLAU	10	0.119	0.0216	NA	NA
DZIP3	3	-0.119	0.0216	-0.191	0.0054
RALY	20	-0.119	0.0216	-0.093	0.1794
APAF1	12	0.119	0.0216	0.534	0
SARS2	19	0.119	0.0217	NA	NA
ELK1	X	-0.119	0.0217	0.250	0.0002
RBM17P4	15	-0.119	0.0218	NA	NA
ACTG1P11	Y	0.119	0.0218	NA	NA
RAB13	1	0.119	0.0219	-0.170	0.0136
PPFIA4	1	-0.119	0.0220	0.306	6.11E-06
RPL23P2	21	0.119	0.0220	NA	NA
AHCYL2	7	0.119	0.0220	NA	NA
TUBE1	6	-0.119	0.0220	NA	NA
SBNO2	19	0.118	0.0221	NA	NA
IPCEF1	6	-0.118	0.0221	NA	NA
FAM167A	8	-0.118	0.0222	NA	NA
KIZ	20	-0.118	0.0222	NA	NA
PDCL	9	-0.118	0.0222	-0.011	0.8771
FAM60A	12	0.118	0.0223	0.005	0.9474
TIMM8B	11	-0.118	0.0223	0.067	0.3301
C16orf87	16	-0.118	0.0224	NA	NA
CXorf21	X	0.118	0.0224	0.149	0.0300
ARPC1B	7	-0.118	0.0225	0.239	0.0005
UBE2E3	2	-0.118	0.0225	-0.225	0.0010
HSPD1P3	8	0.118	0.0225	NA	NA

NUP54	4	-0.118	0.0225	-0.027	0.6990
CYCS	7	0.118	0.0229	-0.133	0.0537
CD300A	17	-0.118	0.0230	0.131	0.0584
FAM105A	5	-0.118	0.0231	0.620	0
TAPT1-AS1	4	0.117	0.0233	NA	NA
LRRC47	1	-0.117	0.0233	0.146	0.0344
IGLC6	22	0.117	0.0233	NA	NA
GTF2E2	8	0.117	0.0234	0.169	0.0142
GPR153	1	0.117	0.0235	-0.067	0.3360
NEK8	17	0.117	0.0235	NA	NA
ECE1	1	0.117	0.0235	0.113	0.1032
TMEM38A	19	0.117	0.0235	NA	NA
LYL1	19	0.117	0.0236	-0.211	0.0020
SLC28A3	9	-0.117	0.0238	0.043	0.5303
TPCN1	12	-0.117	0.0238	0.451	5.98E-12
LINC00865	10	0.117	0.0239	NA	NA
ALDH6A1	14	-0.117	0.0239	-0.206	0.0026
IGHV3-7	14	0.117	0.0240	NA	NA
DPY19L2P1	7	0.117	0.0240	NA	NA
SH3BP5-AS1	3	0.117	0.0241	NA	NA
CFLAR	2	0.117	0.0241	0.379	1.36E-08
KIAA0040	1	0.117	0.0243	0.099	0.1514
PRLR	5	0.117	0.0243	NA	NA
C10orf12	10	-0.117	0.0244	NA	NA
NDUFA2	5	-0.116	0.0244	0.018	0.7970
CDCA8	1	-0.116	0.0245	-0.226	0.0010
ABHD17AP4	22	0.116	0.0245	NA	NA
NSD1	5	0.116	0.0246	0.114	0.0975
AHNAK2	14	0.116	0.0247	NA	NA
THOP1	19	0.116	0.0248	NA	NA
SPRYD7	13	-0.116	0.0249	NA	NA
TMOD2	15	0.116	0.0249	NA	NA
TNFRSF17	16	0.116	0.0250	-0.234	0.0006
SLAIN1	13	-0.116	0.0250	NA	NA
TMEM156	4	0.116	0.0251	-0.119	0.0837
LY75	2	0.116	0.0251	0.199	0.0036
HMGN2P7	3	-0.116	0.0251	NA	NA
ANKH	5	-0.116	0.0251	0.014	0.8454
NPNT	4	0.116	0.0253	NA	NA
MAP4K4	2	0.116	0.0253	0.222	0.0012
ZSCAN16	6	-0.116	0.0253	NA	NA
ZFP36L2	2	0.116	0.0254	0.091	0.1903
YEATS2	3	0.116	0.0254	-0.023	0.7451
SMIM13	6	-0.116	0.0254	NA	NA
ABHD4	14	-0.116	0.0255	-0.101	0.1456

HSPA4	5	-0.116	0.0256	0.010	0.8798
EEF1A1P28	7	0.115	0.0257	NA	NA
LETM1	4	-0.115	0.0258	-0.018	0.7915
WIPI1	17	-0.115	0.0258	0.112	0.1032
RNPC3	1	0.115	0.0258	NA	NA
RPS27L	15	-0.115	0.0258	0.085	0.2184
RPL34P34	19	0.115	0.0259	NA	NA
NHP2P1	10	-0.115	0.0260	NA	NA
TEAD4	12	0.115	0.0261	NA	NA
ADARB1	21	0.115	0.0261	0.068	0.3290
RWDD2A	6	-0.115	0.0262	NA	NA
XAF1	17	0.115	0.0262	NA	NA
PIK3CD-AS1	1	0.115	0.0262	NA	NA
CD69	12	-0.115	0.0263	-0.093	0.1779
TMEM132A	11	0.115	0.0263	NA	NA
ARHGAP25	2	0.115	0.0263	0.172	0.0126
MED26	19	-0.115	0.0263	NA	NA
TPGS2	18	-0.115	0.0263	NA	NA
SSR2	1	-0.115	0.0265	0.197	0.0041
ZSCAN18	19	-0.115	0.0265	NA	NA
TXNDC5	6	0.115	0.0267	-0.286	2.50E-05
FABP5P3	7	0.115	0.0267	NA	NA
ADCK4	19	-0.115	0.0267	NA	NA
MYH6	14	0.115	0.0267	NA	NA
GALK2	15	-0.115	0.0269	0.015	0.8337
FAM53B	10	0.115	0.0269	0.122	0.0778
AMMECR1	X	-0.115	0.0270	-0.009	0.8982
EXD3	9	-0.114	0.0271	NA	NA
LRP6	12	0.114	0.0272	-0.134	0.0519
IMPDH1P10	2	0.114	0.0272	NA	NA
PLEKHM1P	17	-0.114	0.0272	NA	NA
DHX30	3	-0.114	0.0273	-0.018	0.7984
RGS6	14	-0.114	0.0274	NA	NA
PPIF	10	-0.114	0.0275	0.167	0.0153
AMOTL1	11	0.114	0.0276	NA	NA
CLSPN	1	0.114	0.0276	NA	NA
SNX10	7	0.114	0.0277	0.137	0.0470
NOL7	6	-0.114	0.0278	-0.042	0.5477
CHPF2	7	-0.114	0.0278	NA	NA
DNAH14	1	0.114	0.0278	NA	NA
OXA1L	14	-0.114	0.0279	0.274	5.34E-05
RPL3P12	X	0.114	0.0279	NA	NA
AMT	3	-0.114	0.0279	-0.081	0.2422
CMPK1	1	-0.114	0.0280	NA	NA
ECH1	19	-0.114	0.0280	-0.137	0.0473

ZNF121	19	-0.114	0.0280	NA	NA
DEPDC4	12	0.114	0.0281	NA	NA
CD9	12	0.114	0.0282	0.098	0.1558
IRF8	16	-0.114	0.0282	0.053	0.4412
DNAJB6	7	-0.114	0.0282	-0.039	0.5703
SGOL1-AS1	3	-0.114	0.0283	NA	NA
ANO10	3	0.114	0.0283	NA	NA
HSPA5	9	-0.114	0.0283	0.053	0.4395
MTTP	4	0.114	0.0284	NA	NA
HHAT	1	-0.114	0.0284	-0.171	0.0130
FBXL19	16	0.114	0.0284	NA	NA
DET1	15	0.114	0.0284	0.059	0.3943
ZNF569	19	-0.113	0.0284	NA	NA
GDF11	12	-0.113	0.0284	NA	NA
LINC00342	2	0.113	0.0285	NA	NA
QPCTL	19	-0.113	0.0286	NA	NA
CEP250	20	0.113	0.0287	NA	NA
SECISBP2	9	0.113	0.0288	0.058	0.3985
MYC	8	0.113	0.0289	-0.133	0.0545
EEF1A1P8	3	0.113	0.0290	NA	NA
TBC1D22A	22	0.113	0.0290	0.179	0.0092
DNAJB11	3	-0.113	0.0290	NA	NA
MEA1	6	-0.113	0.0290	-0.207	0.0025
IGFBP4	17	0.113	0.0291	-0.171	0.0129
ATP8B1	18	-0.113	0.0291	-0.057	0.4087
PODXL2	3	0.113	0.0293	NA	NA
FAM160A1	4	0.113	0.0294	NA	NA
TCEA1P4	9	-0.113	0.0294	NA	NA
HES1	3	0.113	0.0295	-0.201	0.0034
NME3	16	-0.113	0.0295	NA	NA
HADHA	2	-0.113	0.0296	0.176	0.0102
IRGQ	19	-0.113	0.0296	NA	NA
RPL7P19	5	0.113	0.0296	NA	NA
ADTRP	6	0.113	0.0297	NA	NA
RPSAP14	X	0.113	0.0298	NA	NA
DNLZ	9	0.113	0.0298	NA	NA
IST1	16	0.112	0.0298	NA	NA
FAM160A2	11	-0.112	0.0299	NA	NA
AJUBA	14	-0.112	0.0299	NA	NA
LHFP	13	0.112	0.0299	-0.135	0.0504
KCTD3	1	0.112	0.0300	-0.094	0.1718
AMZ2	17	-0.112	0.0300	0.065	0.3501
RPL35AP35	17	-0.112	0.0301	NA	NA
PSTPIP2	18	-0.112	0.0303	0.224	0.0010
KIF13B	8	-0.112	0.0304	0.205	0.0028

SUB1P3	16	0.112	0.0304	NA	NA
DYNC1LI2	16	-0.112	0.0304	0.319	2.23E-06
PRKX	X	-0.112	0.0304	0.295	1.33E-05
RPL7AP3	14	0.112	0.0305	NA	NA
PSMA6P1	Y	0.112	0.0305	NA	NA
FSTL3	19	0.112	0.0308	NA	NA
SLC45A3	1	0.112	0.0308	NA	NA
RGMB	5	0.112	0.0308	NA	NA
CAMSAP2	1	0.112	0.0310	NA	NA
NXT2	X	-0.112	0.0311	-0.099	0.1535
CYP51A1P2	13	-0.112	0.0311	NA	NA
MYL12BP1	15	0.112	0.0311	NA	NA
OSGIN2	8	-0.112	0.0312	0.185	0.0069
RPL19P20	X	0.112	0.0313	NA	NA
PLAC8	4	0.111	0.0314	-0.058	0.4004
UCN	2	-0.111	0.0314	0.006	0.9338
FUT8	14	0.111	0.0314	-0.201	0.0034
GSTP1	11	-0.111	0.0314	0.048	0.4894
ZNF641	12	0.111	0.0315	NA	NA
RRM2P3	X	0.111	0.0315	NA	NA
TFEB	6	0.111	0.0315	0.102	0.1403
XPOT	12	-0.111	0.0315	0.043	0.5346
C14orf105	14	0.111	0.0315	NA	NA
MRPL13	8	-0.111	0.0316	-0.004	0.9535
ZNF649	19	-0.111	0.0316	NA	NA
NME4	16	0.111	0.0316	-0.365	4.57E-08
PGAM1P7	X	0.111	0.0316	NA	NA
IGLV1-51	22	0.111	0.0316	NA	NA
CHCHD4	3	0.111	0.0317	NA	NA
ZNF680	7	-0.111	0.0318	NA	NA
TPBG	6	-0.111	0.0319	-0.119	0.0856
ZNF248	10	0.111	0.0320	0.017	0.8116
CUL7	6	-0.111	0.0320	NA	NA
UBXN6	19	-0.111	0.0321	NA	NA
SPSB3	16	-0.111	0.0321	0.117	0.0907
TRIM13	13	0.111	0.0321	0.034	0.6285
TSGA10	2	-0.111	0.0323	NA	NA
FHL2	2	-0.111	0.0323	-0.304	6.67E-06
CD4	12	0.111	0.0323	0.739	0
EDN1	6	0.111	0.0323	NA	NA
C19orf52	19	-0.111	0.0323	NA	NA
WDR62	19	-0.111	0.0324	NA	NA
PLEKHA8	7	0.111	0.0324	NA	NA
RFNG	17	-0.111	0.0325	0.074	0.2869
ABCC5	3	0.111	0.0325	0.010	0.8807

GFM2	5	-0.111	0.0325	NA	NA
CDH24	14	-0.111	0.0326	NA	NA
SWT1	1	0.111	0.0326	NA	NA
GPR150	5	-0.111	0.0326	NA	NA
PLS1	3	0.111	0.0326	-0.274	5.63E-05
ZFP30	19	-0.111	0.0326	-0.065	0.3506
LONP1	19	-0.111	0.0327	NA	NA
C7orf26	7	-0.111	0.0327	0.034	0.6231
RABGAP1L	1	0.111	0.0327	0.229	0.0008
CHST12	7	-0.111	0.0328	-0.260	0.0001
SIKE1	1	0.111	0.0328	NA	NA
STRBP	9	-0.111	0.0329	NA	NA
XKR9	8	-0.110	0.0329	NA	NA
IGLC7	22	0.110	0.0329	NA	NA
ADRB1	10	-0.110	0.0329	NA	NA
SLC25A26	3	-0.110	0.0329	NA	NA
HCAR2	12	0.110	0.0330	NA	NA
TANC2	17	-0.110	0.0331	NA	NA
RPS2P46	17	-0.110	0.0331	NA	NA
VCL	10	0.110	0.0331	0.168	0.0143
PHLPP1	18	-0.110	0.0332	NA	NA
SUGP2	19	-0.110	0.0332	NA	NA
UBE2MP1	16	-0.110	0.0332	NA	NA
MSTO2P	1	-0.110	0.0333	NA	NA
PCTP	17	-0.110	0.0333	0.036	0.6047
MBD2	18	-0.110	0.0334	0.285	2.72E-05
KIN	10	0.110	0.0335	0.190	0.0057
YBEY	21	-0.110	0.0336	NA	NA
TMEM200A	6	0.110	0.0336	NA	NA
POU5F1P4	1	0.110	0.0337	NA	NA
IGLC3	22	0.110	0.0338	NA	NA
RPL37P23	19	0.110	0.0339	NA	NA
ANAPC1	2	-0.110	0.0340	0.003	0.9644
PPP1R11P1	1	0.110	0.0341	NA	NA
RPS20P24	9	0.110	0.0341	NA	NA
IFNG	12	-0.110	0.0343	-0.138	0.0459
TTC38	22	-0.110	0.0343	NA	NA
CNR1	6	0.110	0.0344	-0.201	0.0034
FAM212B-AS1	1	-0.110	0.0344	NA	NA
NACAP3	12	0.109	0.0346	NA	NA
C10orf128	10	0.109	0.0346	NA	NA
DARS-AS1	2	0.109	0.0346	NA	NA
AMZ2P1	17	-0.109	0.0347	NA	NA
KLF13	15	0.109	0.0348	NA	NA

NPM1P22	13	0.109	0.0348	NA	NA
SLC44A1	9	0.109	0.0348	NA	NA
RIT1	1	0.109	0.0348	0.069	0.3197
SRIP3	Y	-0.109	0.0350	NA	NA
RAPGEF1	9	0.109	0.0350	0.111	0.1076
CAPN2	1	0.109	0.0350	0.427	8.87E-11
RCL1	9	-0.109	0.0351	-0.306	5.99E-06
C1orf162	1	0.109	0.0351	NA	NA
ZNF135	19	-0.109	0.0351	NA	NA
SERBP1P5	4	-0.109	0.0352	NA	NA
AIM2	1	0.109	0.0352	-0.047	0.4947
POC1A	3	-0.109	0.0352	NA	NA
TMEM67	8	-0.109	0.0353	NA	NA
FA2H	16	0.109	0.0353	NA	NA
FAM3C	7	-0.109	0.0353	-0.282	3.17E-05
DDN	12	0.109	0.0354	NA	NA
SLAMF1	1	0.109	0.0354	0.171	0.0129
FAM199X	X	-0.109	0.0355	NA	NA
TMEM107	17	-0.109	0.0355	NA	NA
RPL6P27	18	-0.109	0.0355	NA	NA
DEAF1	11	0.109	0.0357	NA	NA
FAM129B	9	-0.109	0.0357	NA	NA
RGS19	20	-0.109	0.0358	0.300	9.45E-06
QPCT	2	-0.109	0.0358	0.442	1.64E-11
STK17B	2	-0.109	0.0359	0.402	1.38E-09
ATG13	11	-0.109	0.0361	NA	NA
NME9	3	-0.109	0.0361	NA	NA
HNRNPLP1	6	0.109	0.0361	NA	NA
RNF24	20	-0.109	0.0362	0.175	0.0109
SEPT10	2	0.109	0.0362	NA	NA
PTOV1	19	-0.108	0.0363	-0.083	0.2314
ALG13	X	0.108	0.0365	-0.064	0.3571
FAM219A	9	0.108	0.0365	NA	NA
NPM1P46	2	0.108	0.0366	NA	NA
NLK	17	0.108	0.0367	-0.167	0.0150
CD44	11	0.108	0.0367	0.472	4.27E-13
NOL8P1	4	0.108	0.0367	NA	NA
ELL	19	-0.108	0.0367	NA	NA
JUP	17	0.108	0.0369	-0.068	0.3242
CYBRD1	2	-0.108	0.0370	-0.152	0.0272
FTSJ2	7	-0.108	0.0370	0.187	0.0065
PRKRIRP3	10	-0.108	0.0371	NA	NA
ZNF277	7	0.108	0.0371	NA	NA
IPO7	11	0.108	0.0371	0.139	0.0435
SHOC2	10	-0.108	0.0373	0.013	0.8561

ZNF653	19	0.108	0.0375	NA	NA
HCFC1R1	16	0.108	0.0376	-0.101	0.1427
AFG3L2P1	8	-0.108	0.0376	NA	NA
RPL15P20	16	0.108	0.0376	NA	NA
KCNA1	12	-0.108	0.0377	NA	NA
RHOBTB3	5	-0.108	0.0377	-0.365	4.75E-08
IGLC2	22	0.108	0.0378	NA	NA
TNKS2	10	-0.108	0.0379	0.266	9.13E-05
LRFN4	11	0.108	0.0380	NA	NA
PHB2	12	0.107	0.0380	0.028	0.6889
SPTLC1P5	13	-0.107	0.0380	NA	NA
RPL3P10	8	0.107	0.0380	NA	NA
TBCA	5	0.107	0.0381	0.141	0.0411
GAPDHP69	13	0.107	0.0381	NA	NA
SREK1IP1	5	0.107	0.0382	NA	NA
AUH	9	-0.107	0.0383	-0.156	0.0235
SLC16A1	1	0.107	0.0383	-0.171	0.0128
PUS7	7	0.107	0.0384	-0.043	0.5362
KCNMB3P1	22	-0.107	0.0384	NA	NA
PIGBOS1	15	-0.107	0.0385	NA	NA
UBA52P3	14	0.107	0.0386	NA	NA
KIAA1462	10	0.107	0.0386	-0.273	5.79E-05
PLEK	2	0.107	0.0388	-0.151	0.0287
RPL7L1P9	2	0.107	0.0389	NA	NA
MAF1	8	-0.107	0.0389	NA	NA
SLC3A2	11	-0.107	0.0392	0.081	0.2423
MORF4L1	15	-0.107	0.0393	0.055	0.4297
MTRF1LP1	11	0.107	0.0393	NA	NA
MTX1P1	1	-0.107	0.0393	NA	NA
MGST3	1	0.107	0.0394	0.048	0.4867
RPS12P26	15	0.107	0.0394	NA	NA
HGS	17	-0.107	0.0395	0.045	0.5193
RPL31P63	X	0.107	0.0396	NA	NA
FANCM	14	-0.106	0.0398	NA	NA
SPATA3-AS1	2	0.106	0.0398	NA	NA
PPP2R4	9	0.106	0.0399	-0.021	0.7669
MTND2P28	1	-0.106	0.0399	NA	NA
AKR1B1P1	1	-0.106	0.0399	NA	NA
KBTBD8	3	0.106	0.0400	NA	NA
RBMX2	X	-0.106	0.0400	0.146	0.0337
SMG1P4	16	-0.106	0.0401	NA	NA
SOCS2	12	0.106	0.0403	-0.267	8.84E-05
RNF180	5	0.106	0.0403	NA	NA
AAGAB	15	-0.106	0.0404	NA	NA
HOXB3	17	0.106	0.0405	-0.128	0.0635

SEPT10P1	8	0.106	0.0407	NA	NA
YKT6	7	-0.106	0.0407	0.160	0.0202
HECA	6	-0.106	0.0409	0.247	0.0003
MDFIC	7	0.106	0.0410	0.289	2.04E-05
ZIK1	19	-0.106	0.0411	NA	NA
CYSLTR1	X	0.106	0.0411	-0.115	0.0953
SLC2A3P4	8	0.106	0.0412	NA	NA
STARD13	13	0.106	0.0414	-0.008	0.9067
RPL34P18	8	0.106	0.0414	NA	NA
PIFO	1	-0.106	0.0415	NA	NA
GMEB1	1	0.106	0.0415	NA	NA
FBXL5	4	-0.106	0.0415	0.401	1.51E-09
MIB1	18	-0.106	0.0415	NA	NA
RAPGEF5	7	0.106	0.0416	NA	NA
C9orf41	9	-0.106	0.0417	NA	NA
FOXN3P1	X	0.106	0.0417	NA	NA
SLC41A2	12	0.105	0.0419	NA	NA
MGAT3	22	0.105	0.0419	NA	NA
TPRG1	3	0.105	0.0419	NA	NA
GEMIN8P4	1	-0.105	0.0420	NA	NA
TGM2	20	0.105	0.0420	-0.121	0.0786
SH3PXD2B	5	0.105	0.0420	NA	NA
PCSK4	19	-0.105	0.0422	NA	NA
HIPK1-AS1	1	0.105	0.0422	NA	NA
DTX1	12	-0.105	0.0422	NA	NA
HSPD1P6	3	0.105	0.0422	NA	NA
HMGN2P3	16	-0.105	0.0422	NA	NA
JMJD4	1	-0.105	0.0422	-0.078	0.2564
FAM91A3P	1	-0.105	0.0423	NA	NA
FANCF	11	0.105	0.0423	-0.029	0.6732
SLC30A7	1	-0.105	0.0424	NA	NA
SH3KBP1	X	0.105	0.0425	NA	NA
OSBPL7	17	-0.105	0.0425	NA	NA
RPS15AP38	22	0.105	0.0425	NA	NA
FABP3	1	0.105	0.0425	0.000	0.9964
IGSF10	3	0.105	0.0426	NA	NA
NID1	1	-0.105	0.0426	0.367	3.92E-08
TULP2	19	0.105	0.0427	NA	NA
UNC13D	17	-0.105	0.0427	NA	NA
TMEM129	4	-0.105	0.0428	NA	NA
SURF4	9	-0.105	0.0428	NA	NA
ACTBP11	1	-0.105	0.0430	NA	NA
MRPL39	21	-0.105	0.0431	-0.006	0.9294
HHLA2	3	0.105	0.0435	NA	NA
VPS36	13	-0.105	0.0435	NA	NA

AQP9	15	0.105	0.0435	0.436	3.37E-11
CARM1	19	0.105	0.0436	-0.074	0.2841
GRHPR	9	0.105	0.0436	0.008	0.9104
TUBBP2	13	-0.105	0.0437	NA	NA
SLC16A7	12	-0.104	0.0437	0.260	0.0001
POU5F1P3	12	0.104	0.0439	NA	NA
RPL7P59	7	0.104	0.0439	NA	NA
RPL3P6	5	0.104	0.0440	NA	NA
ATIC	2	0.104	0.0440	-0.090	0.1950
EFNA4	1	0.104	0.0441	-0.151	0.0288
CYP1B1-AS1	2	0.104	0.0441	NA	NA
IGSF8	1	0.104	0.0442	NA	NA
PDE4C	19	-0.104	0.0442	0.043	0.5300
FAM27C	9	0.104	0.0442	NA	NA
NPM1P21	8	0.104	0.0442	NA	NA
ZDBF2	2	-0.104	0.0444	NA	NA
TRIO	5	0.104	0.0444	-0.153	0.0266
GOLGA6L9	15	-0.104	0.0446	NA	NA
MAD1L1	7	-0.104	0.0446	NA	NA
MFSD6	2	-0.104	0.0446	NA	NA
LINC01320	2	0.104	0.0447	NA	NA
KIAA1524	3	-0.104	0.0448	NA	NA
CLIP2	7	0.104	0.0448	NA	NA
LMTK2	7	0.104	0.0448	-0.100	0.1479
BIN2	12	0.104	0.0449	0.322	1.79E-06
SCIN	7	0.104	0.0450	0.019	0.7793
BTK	X	0.104	0.0450	-0.085	0.2162
IRF3	19	-0.104	0.0450	0.065	0.3448
PET117	20	-0.104	0.0451	NA	NA
FIRRE	X	0.104	0.0453	NA	NA
CDC14B	9	0.104	0.0453	0.076	0.2709
TMEM263	12	-0.104	0.0454	NA	NA
CHCHD5	2	-0.104	0.0454	NA	NA
NDUFAF3	3	0.104	0.0454	NA	NA
FOXM1	12	-0.104	0.0455	-0.101	0.1452
CDKN2D	19	0.104	0.0456	-0.044	0.5237
PRR11	17	-0.104	0.0456	0.011	0.8772
PDE4B	1	0.104	0.0456	0.212	0.0020
MIA	19	0.103	0.0458	NA	NA
POU5F1	6	0.103	0.0458	0.067	0.3357
FRMD8	11	-0.103	0.0459	NA	NA
NUDT16L1	16	-0.103	0.0460	NA	NA
PSMD6	3	-0.103	0.0460	0.094	0.1721
ERCC5	13	-0.103	0.0460	0.216	0.0016
SRP68P3	17	0.103	0.0461	NA	NA

ATP5G3	2	-0.103	0.0461	0.066	0.3431
MED21	12	0.103	0.0462	NA	NA
CD200	3	0.103	0.0462	-0.319	2.23E-06
GPX4	19	0.103	0.0463	0.019	0.7831
MYLK-AS1	3	0.103	0.0463	NA	NA
NECAB1	8	0.103	0.0463	NA	NA
BAX	19	-0.103	0.0463	0.226	0.0010
LMCD1	3	0.103	0.0463	NA	NA
MPRIPP1	3	-0.103	0.0464	NA	NA
RAB18	10	-0.103	0.0464	NA	NA
PYCARD-AS1	16	0.103	0.0464	NA	NA
ST13P6	8	-0.103	0.0464	NA	NA
CTTNBP2NL	1	0.103	0.0466	0.022	0.7501
HMGB1P4	2	0.103	0.0467	NA	NA
TNFAIP6	2	0.103	0.0469	0.224	0.0011
CCNA2	4	-0.103	0.0469	-0.222	0.0012
BCO1	16	0.103	0.0470	NA	NA
C1orf106	1	0.103	0.0470	NA	NA
KIAA1671	22	0.103	0.0470	NA	NA
GLDCP1	4	0.103	0.0471	NA	NA
ZNF195	11	0.103	0.0472	-0.067	0.3301
TMEM237	2	0.103	0.0473	NA	NA
KIF9-AS1	3	-0.103	0.0475	NA	NA
ZNF568	19	-0.103	0.0475	NA	NA
PCAT6	1	-0.103	0.0476	NA	NA
HSD17B4	5	-0.103	0.0476	0.116	0.0941
HKR1	19	-0.103	0.0476	NA	NA
DEK	6	-0.103	0.0476	0.048	0.4898
RHBDL1	16	-0.103	0.0477	NA	NA
TADA2B	4	-0.103	0.0477	NA	NA
EEF1E1	6	0.103	0.0477	-0.008	0.9067
PRKCDBP	11	0.103	0.0477	NA	NA
CEP128	14	0.103	0.0477	NA	NA
MTMR9	8	-0.103	0.0478	0.269	7.79E-05
ANKRD29	18	0.102	0.0479	NA	NA
ANXA2P1	4	0.102	0.0480	0.058	0.4044
RIPK2	8	0.102	0.0480	0.030	0.6632
H3F3A	1	0.102	0.0480	-0.063	0.3640
ABCD2	12	0.102	0.0481	0.140	0.0417
APEX2	X	-0.102	0.0481	NA	NA
SRGN	10	0.102	0.0482	NA	NA
RPL21P134	X	-0.102	0.0482	NA	NA
CD47	3	-0.102	0.0482	0.119	0.0855
GAB1	4	0.102	0.0482	-0.286	2.44E-05
SIRPA	20	0.102	0.0483	0.395	2.60E-09

TMCO1	1	0.102	0.0483	0.153	0.0260
STMN3	20	0.102	0.0484	NA	NA
RPL34P33	19	0.102	0.0485	NA	NA
MED13L	12	0.102	0.0485	NA	NA
ARHGAP11B	15	-0.102	0.0486	NA	NA
NOTCH2	1	0.102	0.0486	0.258	0.0002
SMARCE1P6	2	0.102	0.0486	NA	NA
ACSL3	2	-0.102	0.0487	0.084	0.2269
ZNF816	19	0.102	0.0487	NA	NA
NPM1P19	20	0.102	0.0487	NA	NA
LDHAP2	1	0.102	0.0488	NA	NA
DAAM2	6	0.102	0.0488	NA	NA
CBX3P1	11	0.102	0.0490	NA	NA
SETD3	14	-0.102	0.0490	0.194	0.0046
DGKZP1	13	-0.102	0.0490	NA	NA
HMGB3P24	9	-0.102	0.0491	NA	NA
UBA7	3	-0.102	0.0491	NA	NA
PLEKHG2	19	0.102	0.0492	NA	NA
FAM227B	15	-0.102	0.0492	NA	NA
COPB1	11	-0.102	0.0493	0.171	0.0130
PHF10	6	0.102	0.0493	0.244	0.0004
KISS1R	19	0.102	0.0495	NA	NA
NTNG2	9	0.102	0.0495	NA	NA
GPR34	X	0.102	0.0495	NA	NA
PTPRO	12	0.102	0.0495	0.389	4.97E-09
TLR4	9	0.102	0.0495	0.380	1.22E-08
CMC2	16	-0.102	0.0495	NA	NA
PLD4	14	0.102	0.0495	NA	NA
ZBED3	5	0.102	0.0496	NA	NA
TFR2	7	-0.102	0.0496	-0.352	1.45E-07
PTMAP4	12	0.102	0.0497	NA	NA
TMED4	7	-0.102	0.0497	NA	NA
ATG16L2	11	-0.102	0.0497	NA	NA
KIF4A	X	-0.102	0.0498	-0.238	0.0005
LRP10	14	-0.102	0.0498	0.105	0.1277
ZNF25	10	-0.102	0.0499	NA	NA
AP1S3	2	-0.102	0.0500	NA	NA
UPB1	22	0.102	0.0500	NA	NA
LMAN1	18	-0.102	0.0500	-0.087	0.2105
ZFP36L1	14	-0.102	0.0500	0.341	3.69E-07
PTPRC	1	0.102	0.0501	0.308	5.18E-06
EIF3LP1	14	-0.102	0.0501	NA	NA
FAM129A	1	0.102	0.0501	0.337	5.49E-07
AMD1	6	-0.101	0.0502	-0.120	0.0830
BOLA3-AS1	2	0.101	0.0503	NA	NA

GLE1	9	-0.101	0.0504	NA	NA
ZNF264	19	-0.101	0.0506	0.193	0.0049
PCGF3	4	0.101	0.0506	0.127	0.0657
SERTAD2	2	0.101	0.0506	-0.011	0.8747
NFE2L3	7	0.101	0.0507	0.119	0.0833
MLLT3	9	-0.101	0.0507	-0.120	0.0826
IGLV1-44	22	0.101	0.0507	NA	NA
RPS18P13	19	0.101	0.0508	NA	NA
ACSL5	10	0.101	0.0508	0.221	0.0013
ZNF611	19	-0.101	0.0509	0.031	0.6578
TPT1P6	6	0.101	0.0509	NA	NA
SELPLG	12	0.101	0.0509	0.515	1.11E-15
MICU1	10	0.101	0.0510	NA	NA
CLPB	11	-0.101	0.0510	NA	NA
TMED6	16	-0.101	0.0511	NA	NA
PTPN1	20	0.101	0.0513	0.168	0.0149
PHBP11	1	0.101	0.0513	NA	NA
ANXA3	4	0.101	0.0514	0.061	0.3788
DLGAP1-AS2	18	0.101	0.0516	NA	NA
RALGPS2	1	0.101	0.0517	-0.170	0.0133
SCAP	3	-0.101	0.0517	0.073	0.2923
C11orf63	11	0.101	0.0519	0.082	0.2373
RPS6KA5	14	0.101	0.0519	0.237	0.0005
CASP9	1	-0.101	0.0519	0.033	0.6386
FBXW7	4	0.101	0.0519	0.279	4.01E-05
EP300	22	0.101	0.0519	0.186	0.0067
TBL3	16	-0.101	0.0519	NA	NA
SYNPO2	4	-0.101	0.0520	-0.001	0.9840
ZNF736	7	-0.101	0.0520	NA	NA
QPRT	16	-0.101	0.0520	NA	NA
CCDC13	3	0.101	0.0521	NA	NA
RPL23	17	-0.101	0.0522	0.038	0.5856
LEAP2	5	0.101	0.0522	NA	NA
ACTL10	20	-0.101	0.0523	NA	NA
ANKS3	16	-0.101	0.0524	NA	NA
DGKE	17	-0.101	0.0524	-0.007	0.9231
SORT1	1	-0.100	0.0525	0.403	1.26E-09
SMEK1	14	-0.100	0.0525	0.216	0.0016
ABRACL	6	-0.100	0.0526	NA	NA
NPM1P6	8	-0.100	0.0526	NA	NA
AICDA	12	0.100	0.0526	NA	NA
B3GNTL1	17	0.100	0.0528	NA	NA
TROAP	12	-0.100	0.0529	NA	NA
WDFY2	13	0.100	0.0529	NA	NA
RAB42P1	14	0.100	0.0529	NA	NA

NOP9	14	-0.100	0.0530	NA	NA
ZNF324B	19	-0.100	0.0531	NA	NA
ARPC3	12	0.100	0.0531	0.307	5.62E-06
ZWILCH	15	-0.100	0.0533	-0.305	6.66E-06
EEF1A1P25	3	0.100	0.0533	NA	NA
ARHGEF7	13	-0.100	0.0533	0.113	0.1030
FLOT2	17	0.100	0.0535	0.200	0.0034
LINC00426	13	0.100	0.0535	NA	NA
HMGA1P3	12	0.100	0.0535	NA	NA
SEMA7A	15	-0.100	0.0537	NA	NA
LCMT1-AS2	16	-0.100	0.0539	NA	NA
DROSHA	5	-0.100	0.0540	NA	NA
GAPDHP1	X	-0.100	0.0540	NA	NA
MYBPC2	19	0.100	0.0540	NA	NA
FAM72A	1	-0.100	0.0541	NA	NA
RPS3AP22	5	0.100	0.0542	NA	NA
SRIP2	X	-0.100	0.0543	NA	NA
CLK1	2	0.100	0.0544	0.122	0.0759
GNPNAT1	14	-0.100	0.0544	NA	NA
CCNE1	19	-0.100	0.0544	-0.090	0.1908
MAP3K15	X	0.100	0.0544	0.019	0.7840
KHSRP	19	-0.100	0.0544	0.010	0.8828
ARAP1	11	-0.100	0.0544	NA	NA
TBXAS1	7	0.100	0.0546	0.295	1.36E-05
TMEM109	11	0.100	0.0546	0.085	0.2197
KTI12	1	-0.100	0.0546	NA	NA
C16orf13	16	-0.100	0.0547	NA	NA
HAUS1P1	5	0.100	0.0547	NA	NA
SHB	9	0.100	0.0547	0.071	0.3069
SYTL3	6	-0.100	0.0548	NA	NA
PARP14	3	0.100	0.0549	NA	NA
HCP5	6	0.099	0.0549	NA	NA
RPL17P43	17	0.099	0.0549	NA	NA
ITGA6	2	0.099	0.0549	0.115	0.0966
PCP4L1	1	0.099	0.0550	NA	NA
KDM2A	11	0.099	0.0550	NA	NA
TEKT4	2	-0.099	0.0550	NA	NA
AFTPH	2	-0.099	0.0551	0.148	0.0316
ALDOC	17	-0.099	0.0552	-0.076	0.2749
PPP1R21	2	-0.099	0.0553	NA	NA
KIF23	15	-0.099	0.0553	NA	NA
ATP1A1-AS1	1	0.099	0.0554	NA	NA
IFIH1	2	0.099	0.0554	0.236	0.0005
ELMO1	7	0.099	0.0555	0.057	0.4063
GATAD2B	1	-0.099	0.0556	NA	NA

DIABLO	12	0.099	0.0558	0.093	0.1795
DDB2	11	-0.099	0.0558	0.152	0.0276
SRSF6	20	-0.099	0.0560	NA	NA
FLI1	11	0.099	0.0560	0.159	0.0211
STK40	1	-0.099	0.0560	NA	NA
VN1R1	19	-0.099	0.0561	NA	NA
RASSF7	11	0.099	0.0562	0.094	0.1739
RPS2P4	14	-0.099	0.0562	NA	NA
DDX3X	X	-0.099	0.0563	0.041	0.5520
ADAM19	5	0.099	0.0564	0.205	0.0028
CUL5	11	-0.099	0.0564	0.156	0.0237
MRPS36P1	3	-0.099	0.0566	NA	NA
EFNA5	5	0.099	0.0567	NA	NA
C6orf136	6	-0.099	0.0568	NA	NA
IGLC1	22	0.099	0.0568	NA	NA
NET1	10	-0.099	0.0569	-0.097	0.1595
ERBB2	17	-0.099	0.0569	-0.175	0.0109
MRPL51	12	-0.099	0.0570	NA	NA
EPRS	1	-0.099	0.0571	0.094	0.1730
KCMF1	2	-0.099	0.0571	0.219	0.0014
PDE4A	19	-0.099	0.0572	0.309	4.62E-06
ZNF565	19	-0.099	0.0573	NA	NA
TFAP2A	6	0.099	0.0573	NA	NA
MBP	18	0.099	0.0573	0.242	0.0004
CD160	1	-0.098	0.0575	-0.104	0.1328
UCK2	1	0.098	0.0575	-0.255	0.0002
BCL2L11	2	-0.098	0.0577	-0.059	0.3925
GRAMD1C	3	0.098	0.0577	-0.293	1.51E-05
AP2A2	11	0.098	0.0577	0.126	0.0679
NELFA	4	0.098	0.0577	NA	NA
DLGAP5	14	-0.098	0.0578	NA	NA
HSPA9P1	2	-0.098	0.0578	NA	NA
GAPDHP63	6	0.098	0.0579	NA	NA
RPL21P119	16	-0.098	0.0580	NA	NA
TRMT5	14	-0.098	0.0581	-0.030	0.6609
ATP11A	13	0.098	0.0581	0.457	2.83E-12
BIK	22	0.098	0.0581	0.101	0.1453
LDHAP1	4	0.098	0.0581	NA	NA
PTPN2P1	1	0.098	0.0582	NA	NA
TEC	4	-0.098	0.0584	NA	NA
STAM-AS1	10	0.098	0.0584	NA	NA
P2RY10	X	-0.098	0.0585	0.051	0.4582
PCOLCE	7	-0.098	0.0585	NA	NA
DACT3	19	0.098	0.0586	NA	NA
TMEM117	12	0.098	0.0586	NA	NA

FUNDC2	X	-0.098	0.0587	NA	NA
C11orf65	11	-0.098	0.0587	NA	NA
ACTG1	17	-0.098	0.0587	0.214	0.0018
STX12	1	0.098	0.0588	0.406	9.00E-10
BCL2L13	22	0.098	0.0588	0.094	0.1719
SNX18	5	-0.098	0.0588	NA	NA
ZNF134	19	-0.098	0.0588	0.055	0.4243
BRI3	7	0.098	0.0588	NA	NA
HNRNPA1P35	2	0.098	0.0589	NA	NA
PDK2	17	0.098	0.0590	NA	NA
TMEM2	9	-0.098	0.0591	0.301	8.71E-06
SUV39H2	10	-0.098	0.0591	-0.104	0.1310
GNA11	19	0.098	0.0591	-0.086	0.2131
STAG3L4	7	-0.098	0.0592	NA	NA
C1orf123	1	0.098	0.0592	0.203	0.0031
PHBP21	16	0.098	0.0593	NA	NA
LBR	1	0.098	0.0593	0.014	0.8433
SLC39A7	6	-0.098	0.0594	0.012	0.8593
SEMA6A-AS1	5	0.098	0.0594	NA	NA
CCSER2	10	0.098	0.0594	NA	NA
SIN3B	19	-0.098	0.0594	0.285	2.55E-05
TUBB	6	-0.098	0.0595	-0.267	8.71E-05
EBNA1BP2	1	0.098	0.0595	-0.232	0.0007
ZNF853	7	-0.098	0.0595	NA	NA
REL	2	0.098	0.0595	0.070	0.3122
CCSER1	4	-0.098	0.0596	NA	NA
PABPC1P4	12	0.098	0.0596	NA	NA
CBR3	21	0.098	0.0596	0.108	0.1187
KIF6	6	0.098	0.0596	NA	NA
TMEM170A	16	-0.098	0.0598	NA	NA
XPO6	16	-0.098	0.0599	0.242	0.0004
HMGN4	6	0.097	0.0600	0.208	0.0024
ATP5G2	12	-0.097	0.0600	0.068	0.3221
BBS10	12	0.097	0.0600	0.090	0.1952
MCTP2	15	-0.097	0.0601	-0.374	2.04E-08
PPP2R5C	14	0.097	0.0601	0.201	0.0034
FAM49B	8	-0.097	0.0603	0.202	0.0032
ERICH3	1	-0.097	0.0603	NA	NA
E2F2	1	0.097	0.0604	-0.169	0.0141
RNPS1	16	-0.097	0.0604	0.014	0.8357
TCEB2P2	11	0.097	0.0604	NA	NA
NDUFA10	2	-0.097	0.0604	0.164	0.0175
ARHGEF1	19	-0.097	0.0606	0.290	1.88E-05
KRT18P34	3	-0.097	0.0606	NA	NA
PRKAA1	5	-0.097	0.0607	-0.010	0.8836

MTND1P23	1	-0.097	0.0607	NA	NA
DHX8	17	-0.097	0.0608	0.263	0.0001
RPL21P2	20	0.097	0.0609	NA	NA
ANKFY1	17	0.097	0.0610	NA	NA
YBX1P1	14	-0.097	0.0611	NA	NA
ITGA3	17	0.097	0.0612	NA	NA
PAQR4	16	0.097	0.0613	NA	NA
LAT	16	-0.097	0.0613	0.295	1.31E-05
PEG10	7	0.097	0.0613	-0.236	0.0006
PSME2P2	13	0.097	0.0615	NA	NA
RWDD4P2	7	0.097	0.0615	NA	NA
RPEL1	10	0.097	0.0615	NA	NA
RAD54L	1	-0.097	0.0615	-0.284	2.75E-05
MUC12	7	0.097	0.0616	NA	NA
RCN3	19	0.097	0.0616	0.006	0.9261
UCHL5	1	-0.097	0.0617	-0.059	0.3977
CHID1	11	-0.097	0.0617	NA	NA
ECI2	6	-0.097	0.0618	NA	NA
DIP2A	21	-0.097	0.0619	0.069	0.3181
CDKN2C	1	-0.097	0.0619	NA	NA
COX6C	8	-0.097	0.0619	-0.007	0.9174
PRR14L	22	0.097	0.0619	NA	NA
CCNC	6	-0.097	0.0620	0.150	0.0289
ERHP1	7	0.097	0.0620	NA	NA
EID1	15	0.097	0.0621	-0.087	0.2056
IMPACT	18	-0.097	0.0622	0.101	0.1444
LUC7L	16	0.097	0.0624	0.102	0.1411
LINC00937	12	0.097	0.0624	NA	NA
MEST	7	0.097	0.0624	-0.255	0.0002
KIF21B	1	0.097	0.0625	0.158	0.0216
SNAI3	16	0.097	0.0625	NA	NA
LSP1	11	0.096	0.0627	0.369	3.29E-08
RIMKLA	1	-0.096	0.0627	NA	NA
IER5	1	0.096	0.0628	0.117	0.0897
COL18A1	21	-0.096	0.0628	-0.052	0.4515
ANKRD13A	12	0.096	0.0628	NA	NA
ZNF674-AS1	X	0.096	0.0628	NA	NA
ANKRD44	2	0.096	0.0629	NA	NA
PRTFDC1	10	0.096	0.0629	NA	NA
TNFRSF21	6	0.096	0.0630	-0.249	0.0003
KDM3B	5	0.096	0.0631	NA	NA
RPS10P3	9	-0.096	0.0631	NA	NA
NDUFC1	4	0.096	0.0632	0.023	0.7409
GRIN2D	19	0.096	0.0632	NA	NA
PNMA1	14	0.096	0.0632	0.007	0.9218

SPARC	5	0.096	0.0632	-0.004	0.9577
GPLD1	6	0.096	0.0632	-0.162	0.0187
MRPS18AP1	3	0.096	0.0633	NA	NA
ILDR2	1	0.096	0.0633	NA	NA
HSPE1	2	-0.096	0.0634	-0.048	0.4839
ZBTB4	17	-0.096	0.0634	NA	NA
DACT1	14	0.096	0.0634	0.058	0.4025
ZG16B	16	-0.096	0.0634	NA	NA
C12orf74	12	0.096	0.0636	NA	NA
GIMAP1	7	-0.096	0.0637	NA	NA
IMPAD1	8	-0.096	0.0637	NA	NA
CCNB2	15	-0.096	0.0638	-0.280	3.62E-05
MARCH9	12	0.096	0.0638	NA	NA
CXXC5	5	0.096	0.0639	NA	NA
SLC9A7P1	12	0.096	0.0641	NA	NA
ADAMTS12	5	0.096	0.0643	NA	NA
RSBN1	1	0.096	0.0643	0.145	0.0358
NAMPTP1	10	0.096	0.0645	NA	NA
APOL2	22	0.096	0.0646	0.190	0.0057
FAR2	12	-0.096	0.0646	NA	NA
TRAF3IP2	6	-0.096	0.0647	-0.072	0.2974
TERF1P4	X	-0.096	0.0647	NA	NA
CD83	6	0.096	0.0647	0.064	0.3542
RASGRP4	19	0.096	0.0648	NA	NA
KPNA3	13	-0.096	0.0648	0.028	0.6852
CCDC18	1	-0.096	0.0649	NA	NA
NPM1P25	10	0.096	0.0650	NA	NA
MZT1	13	-0.096	0.0650	NA	NA
SPATA9	5	-0.096	0.0651	NA	NA
SLC29A2	11	-0.096	0.0651	NA	NA
ACPP	3	0.096	0.0654	0.360	7.26E-08
FAM171B	2	0.095	0.0655	NA	NA
CLSTN3	12	-0.095	0.0655	-0.032	0.6390
METTL6	3	-0.095	0.0656	NA	NA
ARSG	17	-0.095	0.0656	NA	NA
AP1S1	7	-0.095	0.0658	0.048	0.4901
MTA3	2	0.095	0.0658	NA	NA
HNRNPA1P51	2	0.095	0.0658	NA	NA
PRKACA	19	-0.095	0.0659	0.451	5.45E-12
COG3	13	-0.095	0.0659	NA	NA
LILRB4	19	0.095	0.0660	0.015	0.8341
IFI6	1	0.095	0.0660	-0.316	2.91E-06
SLC9A7	X	0.095	0.0660	-0.279	3.83E-05
P2RX5	17	0.095	0.0661	-0.136	0.0486
TMEM86B	19	-0.095	0.0661	NA	NA

ATF7	12	0.095	0.0662	0.031	0.6571
CCDC51	3	0.095	0.0662	-0.180	0.0088
SH2B1	16	0.095	0.0662	NA	NA
LSM6	4	-0.095	0.0664	0.219	0.0014
RNASE6	14	0.095	0.0665	0.242	0.0004
ARHGEF38	4	0.095	0.0667	NA	NA
HMGA1P6	13	0.095	0.0667	NA	NA
CLCN5	X	0.095	0.0668	0.132	0.0559
SLC30A6	2	0.095	0.0668	NA	NA
H2AFJ	12	0.095	0.0669	-0.168	0.0144
GTF2H2	5	-0.095	0.0670	0.112	0.1040
RDH13	19	-0.095	0.0670	NA	NA
ASS1	9	-0.095	0.0670	NA	NA
ST20	15	0.095	0.0671	NA	NA
FCHSD2	11	0.095	0.0671	0.086	0.2149
RPS11P5	12	0.095	0.0671	NA	NA
NDNL2	15	-0.095	0.0671	NA	NA
RINL	19	-0.095	0.0672	NA	NA
MAN1A2	1	0.095	0.0672	0.150	0.0294
NDUFA4L2	12	0.095	0.0673	NA	NA
RITA1	12	-0.095	0.0673	NA	NA
RPSAP4	14	0.095	0.0673	NA	NA
TARSL2	15	-0.095	0.0673	NA	NA
CD58	1	0.095	0.0674	0.175	0.0109
RANGAP1	22	-0.095	0.0674	NA	NA
MITD1	2	0.095	0.0675	NA	NA
ITIH1	3	-0.095	0.0676	NA	NA
CENPN	16	-0.095	0.0676	-0.291	1.77E-05
ELF4	X	0.095	0.0676	0.360	7.77E-08
ACBD5	10	0.095	0.0677	NA	NA
LMTK3	19	-0.095	0.0679	NA	NA
H1FX	3	0.095	0.0679	0.066	0.3383
KCTD12	13	0.095	0.0679	0.481	1.35E-13
COQ10A	12	0.095	0.0680	NA	NA
RNF169	11	0.095	0.0680	NA	NA
MAP2K2	19	-0.095	0.0680	-0.100	0.1493
RPS23P2	4	0.095	0.0682	NA	NA
UBE2V1	20	-0.095	0.0682	-0.089	0.1967
HSP90AB3P	4	-0.095	0.0683	NA	NA
PPIB	15	-0.094	0.0683	-0.061	0.3795
RPLP1P6	5	0.094	0.0683	NA	NA
DDT	22	0.094	0.0684	0.089	0.1999
PLEKHG3	14	0.094	0.0687	NA	NA
ARHGAP6	X	0.094	0.0687	-0.009	0.8946
CTAGE1	18	0.094	0.0689	NA	NA

XPO4	13	-0.094	0.0689	0.173	0.0117
EFNA3	1	-0.094	0.0689	NA	NA
RHOU	1	0.094	0.0690	NA	NA
PER1	17	-0.094	0.0690	0.087	0.2074
IL18	11	-0.094	0.0694	-0.047	0.4981
C3orf67	3	0.094	0.0695	NA	NA
SLC46A1	17	-0.094	0.0695	NA	NA
AIM1	6	-0.094	0.0695	0.393	3.41E-09
FLII	17	-0.094	0.0695	0.202	0.0031
CCDC85B	11	0.094	0.0695	-0.068	0.3291
BRD4	19	-0.094	0.0696	-0.030	0.6631
JMJD1C	10	-0.094	0.0696	0.209	0.0023
LIMD1-AS1	3	0.094	0.0696	NA	NA
TSPYL1	6	-0.094	0.0697	-0.084	0.2266
SETD6	16	0.094	0.0700	0.058	0.4036
HMGN2P5	15	-0.094	0.0700	NA	NA
ATP10D	4	-0.094	0.0700	0.031	0.6588
MAPK6PS4	8	-0.094	0.0701	NA	NA
ITGB3	17	0.094	0.0702	-0.039	0.5778
ZNF322P1	9	0.094	0.0702	NA	NA
SLC26A1	4	0.094	0.0703	NA	NA
IFI16	1	0.094	0.0703	0.179	0.0091
STYX	14	-0.094	0.0703	NA	NA
SGCB	4	0.094	0.0703	-0.055	0.4296
PRKAR1A	17	-0.094	0.0703	0.168	0.0147
ESF1	20	-0.094	0.0703	0.070	0.3117
MCM6	2	0.094	0.0704	-0.148	0.0319
DTD1	20	-0.094	0.0707	NA	NA
SIDT1	3	-0.094	0.0707	0.051	0.4594
RPS20	8	-0.094	0.0708	0.043	0.5320
C16orf74	16	0.094	0.0709	NA	NA
SH3D21	1	-0.094	0.0709	NA	NA
GLIS2	16	0.094	0.0710	NA	NA
SNORD3B-2	17	0.094	0.0711	NA	NA
EEF1A1P16	12	0.094	0.0711	NA	NA
NPHP4	1	-0.094	0.0711	NA	NA
RPSA	3	-0.094	0.0712	NA	NA
CAPRIN2	12	-0.093	0.0713	NA	NA
RAB8A	19	-0.093	0.0714	0.108	0.1193
WDR83OS	19	-0.093	0.0714	NA	NA
NXPH3	17	0.093	0.0714	NA	NA
FRMD4B	3	0.093	0.0715	0.080	0.2478
APOL6	22	0.093	0.0716	0.261	0.0001
PPP1CC	12	0.093	0.0716	0.081	0.2424
CAPN7	3	-0.093	0.0717	0.182	0.0082

ZNF426	19	-0.093	0.0718	NA	NA
BRD7P2	3	-0.093	0.0718	NA	NA
IL17RA	22	-0.093	0.0718	0.622	0
KRT8P46	4	0.093	0.0719	NA	NA
KLF9	9	-0.093	0.0719	0.244	0.0003
ID3	1	0.093	0.0719	0.054	0.4393
TMC6	17	-0.093	0.0719	0.175	0.0109
LCN10	9	-0.093	0.0721	NA	NA
TBP	6	-0.093	0.0722	0.167	0.0152
TTC13	1	-0.093	0.0722	0.245	0.0003
TAF11	6	-0.093	0.0723	0.011	0.8692
BAP1	3	-0.093	0.0723	-0.081	0.2438
PIKFYVE	2	0.093	0.0723	NA	NA
SATL1	X	0.093	0.0725	0.051	0.4611
ZNF962P	13	0.093	0.0725	NA	NA
VGLL4	3	0.093	0.0725	0.026	0.7100
POLR1A	2	0.093	0.0726	NA	NA
ITSN2	2	0.093	0.0727	0.192	0.0050
GIGYF2	2	-0.093	0.0728	NA	NA
ADRB2	5	0.093	0.0728	0.059	0.3959
RUSC1-AS1	1	-0.093	0.0729	NA	NA
BASP1	5	0.093	0.0729	-0.100	0.1488
OLA1P2	17	0.093	0.0729	NA	NA
PREPL	2	-0.093	0.0731	0.110	0.1112
SLC5A3	21	0.093	0.0731	0.165	0.0165
ASNSP4	8	-0.093	0.0732	NA	NA
NRAS	1	-0.093	0.0732	-0.041	0.5566
GPT2	16	-0.093	0.0732	NA	NA
EDNRB	13	0.093	0.0734	-0.091	0.1903
FCER1G	1	-0.093	0.0735	0.127	0.0646
BTBD2	19	-0.093	0.0735	NA	NA
ACTG1P17	15	-0.093	0.0735	NA	NA
ZNF529	19	-0.093	0.0736	-0.092	0.1825
SLC35A5	3	-0.093	0.0736	0.237	0.0005
IRF2BPL	14	0.093	0.0737	NA	NA
LIN7C	11	-0.093	0.0738	0.085	0.2185
ACTN4P1	4	0.093	0.0738	NA	NA
VPS37B	12	0.093	0.0738	-0.202	0.0032
SENCR	11	0.093	0.0742	NA	NA
IFI44L	1	0.093	0.0742	0.271	6.59E-05
CNOT6	5	-0.093	0.0743	0.030	0.6610
MCTP1	5	-0.092	0.0744	0.232	0.0007
GOLGA6L4	15	-0.092	0.0745	NA	NA
MNDA	1	0.092	0.0746	0.333	7.19E-07
TSEN34	19	0.092	0.0746	0.358	8.93E-08

POLB	8	-0.092	0.0747	0.204	0.0029
TMEM154	4	0.092	0.0748	NA	NA
TOM1	22	-0.092	0.0749	NA	NA
DCHS2	4	0.092	0.0749	NA	NA
ZNF821	16	-0.092	0.0749	NA	NA
CCDC81	11	0.092	0.0750	NA	NA
DCBLD2	3	-0.092	0.0750	-0.189	0.0060
CC2D1A	19	-0.092	0.0750	-0.021	0.7566
NDUFV1	11	-0.092	0.0750	0.085	0.2212
VLDLR-AS1	9	-0.092	0.0750	NA	NA
SLC12A7	5	0.092	0.0751	0.340	4.16E-07
PSMB9	6	0.092	0.0752	0.253	0.0002
FBXO33	14	-0.092	0.0755	NA	NA
RUNX1	21	0.092	0.0755	0.137	0.0463
VPS25	17	-0.092	0.0755	NA	NA
PMS2CL	7	-0.092	0.0757	NA	NA
CPA4	7	0.092	0.0757	NA	NA
DBI	2	-0.092	0.0757	0.145	0.0356
BLCAP	20	-0.092	0.0758	0.145	0.0357
FOXP1	3	0.092	0.0758	0.364	5.34E-08
SSH3	11	-0.092	0.0759	0.073	0.2919
HINT1	5	-0.092	0.0759	0.012	0.8636
MTM1	X	-0.092	0.0760	0.323	1.62E-06
PEX12	17	0.092	0.0760	-0.125	0.0707
SF3B1	2	0.092	0.0760	0.226	0.0010
PLEKHF1	19	-0.092	0.0761	NA	NA
RPS24P17	16	0.092	0.0761	NA	NA
IFI30	19	-0.092	0.0762	0.368	3.72E-08
ZNF608	5	-0.092	0.0762	NA	NA
RPL7P37	10	0.092	0.0762	NA	NA
SGMS1	10	-0.092	0.0763	NA	NA
FABP5P2	13	0.092	0.0763	NA	NA
RGS1	1	-0.092	0.0763	0.106	0.1246
GATC	12	-0.092	0.0765	NA	NA
MARCH8	10	0.092	0.0765	NA	NA
IRS1	2	0.092	0.0765	0.143	0.0382
LIMK2	22	-0.092	0.0765	0.341	3.81E-07
PNKP	19	-0.092	0.0766	-0.156	0.0238
PDXP	22	-0.092	0.0766	NA	NA
ORC3	6	0.092	0.0770	NA	NA
PCED1B-AS1	12	0.092	0.0770	NA	NA
SRSF5	14	-0.092	0.0771	NA	NA
RPS2P6	18	0.092	0.0771	NA	NA
CHAC1	15	-0.092	0.0771	NA	NA
ZNF682	19	-0.092	0.0772	-0.083	0.2305

JAM2	21	-0.092	0.0774	NA	NA
DSC3	18	-0.092	0.0775	NA	NA
MCMBP	10	0.092	0.0775	NA	NA
SLC35E2B	1	-0.091	0.0776	NA	NA
GPR68	14	-0.091	0.0777	-0.014	0.8436
TSPAN12	7	-0.091	0.0778	NA	NA
IQCD	12	-0.091	0.0778	NA	NA
C1orf52	1	-0.091	0.0779	NA	NA
ZHX2	8	0.091	0.0779	-0.047	0.4937
KDM1B	6	0.091	0.0780	NA	NA
HMGB2	4	-0.091	0.0780	-0.097	0.1590
PDE12	3	-0.091	0.0780	NA	NA
BCL2L14	12	0.091	0.0780	NA	NA
MPC1	6	-0.091	0.0781	NA	NA
SLC29A4	7	-0.091	0.0781	NA	NA
FFAR2	19	0.091	0.0782	NA	NA
C10orf55	10	0.091	0.0782	NA	NA
FAM175B	10	0.091	0.0782	NA	NA
SLC52A1	17	-0.091	0.0782	NA	NA
OAT	10	-0.091	0.0784	-0.097	0.1602
PLGRKT	9	-0.091	0.0784	NA	NA
ZNF219	14	0.091	0.0784	-0.195	0.0045
SDSL	12	0.091	0.0786	NA	NA
RBM8B	14	-0.091	0.0787	NA	NA
ANKHD1	5	0.091	0.0787	0.221	0.0012
RGS10	10	0.091	0.0787	0.250	0.0002
CLK4	5	0.091	0.0788	0.149	0.0307
GMPSP1	4	-0.091	0.0788	NA	NA
PEX3	6	-0.091	0.0788	0.079	0.2531
CDH23	10	0.091	0.0791	NA	NA
RPL7AP6	14	-0.091	0.0791	NA	NA
JAG1	20	-0.091	0.0792	0.159	0.0212
ARL8B	3	0.091	0.0792	0.205	0.0028
DHX9P1	13	0.091	0.0793	NA	NA
SNHG17	20	-0.091	0.0794	NA	NA
HSDL2	9	-0.091	0.0796	0.083	0.2283
TMED10	14	-0.091	0.0796	0.132	0.0555
RABGGTB	1	-0.091	0.0797	0.071	0.3064
CHEK1	11	0.091	0.0798	-0.333	7.14E-07
FAM3C2	X	-0.091	0.0798	NA	NA
PDXDC1	16	0.091	0.0801	NA	NA
RAB15	14	0.091	0.0801	NA	NA
DRAM1	12	0.091	0.0801	NA	NA
LINC00094	9	-0.091	0.0802	NA	NA
AZIN1	8	-0.091	0.0802	0.145	0.0357

PSMA1	11	0.091	0.0802	0.124	0.0717
CECR1	22	0.091	0.0803	0.436	3.17E-11
BCLAF1	6	-0.091	0.0803	0.099	0.1533
ARID5B	10	0.091	0.0804	-0.047	0.4927
C17orf75	17	-0.091	0.0804	0.020	0.7713
PA2G4P2	20	0.091	0.0806	NA	NA
HIP1R	12	0.091	0.0806	NA	NA
BCL10	1	0.091	0.0806	0.117	0.0890
CCDC101	16	0.091	0.0806	0.142	0.0398
UBE2SP1	17	-0.091	0.0807	NA	NA
NXPH4	12	0.091	0.0808	0.012	0.8583
TMEM189	20	0.091	0.0808	NA	NA
FAM103A2P	6	-0.091	0.0809	NA	NA
TMEM14A	6	-0.090	0.0810	-0.218	0.0015
PARL	3	-0.090	0.0810	0.256	0.0002
SVIL	10	0.090	0.0810	0.475	2.81E-13
SRD5A1	5	-0.090	0.0811	0.325	1.45E-06
SPAG16	2	0.090	0.0812	-0.235	0.0006
PPA2	4	-0.090	0.0813	0.097	0.1593
NCKAP1	2	0.090	0.0813	-0.083	0.2290
ZNF337	20	-0.090	0.0814	0.157	0.0224
MLX	17	0.090	0.0816	0.364	5.13E-08
CCR1	3	0.089	0.0861	0.477	2.27E-13
SLC16A6	17	-0.088	0.0887	0.318	2.45E-06
CDK5R1	17	-0.088	0.0902	0.349	2.01E-07
BAG3	10	0.087	0.0916	0.383	8.55E-09
TNFAIP2	14	0.087	0.0928	0.330	9.33E-07
TDRD7	9	0.087	0.0932	0.337	5.51E-07
MKNK1	1	0.087	0.0932	0.332	7.77E-07
F2R	5	-0.087	0.0951	-0.354	1.29E-07
FAS	10	-0.086	0.0955	0.339	4.56E-07
NUMB	14	0.086	0.0956	0.407	8.03E-10
MKKS	20	-0.086	0.0957	0.456	3.04E-12
NINJ1	9	-0.086	0.0978	0.375	1.87E-08
KIAA0513	16	-0.085	0.1004	0.486	6.79E-14
SLC27A2	15	-0.085	0.1022	-0.323	1.63E-06
IL11RA	9	0.084	0.1056	0.328	1.08E-06
CD209	19	0.083	0.1081	0.364	5.06E-08
SNN	16	0.083	0.1084	0.445	1.19E-11
ZNF516	18	-0.083	0.1103	0.392	3.76E-09
GLG1	16	-0.083	0.1104	0.393	3.18E-09
MICAL3	22	0.081	0.1167	-0.394	3.10E-09
STX2	12	0.081	0.1183	0.352	1.47E-07
LTB4R	14	0.081	0.1187	0.382	9.33E-09
ZDHHC7	16	-0.081	0.1188	0.424	1.34E-10

HSPA6	1	0.081	0.1192	0.332	7.77E-07
TK2	16	-0.081	0.1200	0.451	5.52E-12
CREBL2	12	-0.080	0.1216	0.469	5.98E-13
LY96	8	0.080	0.1218	0.532	0
NECAP2	1	0.080	0.1227	0.476	2.40E-13
GNPDA1	5	-0.080	0.1229	0.561	0
SGK3	8	-0.080	0.1235	0.368	3.73E-08
HDAC4	2	0.079	0.1281	0.430	6.39E-11
FGL2	7	0.079	0.1284	0.487	6.06E-14
SULT1A2	16	0.078	0.1305	0.433	4.74E-11
TCIRG1	11	-0.078	0.1320	0.361	6.79E-08
ATP6AP1	X	0.078	0.1333	0.394	3.09E-09
CD86	3	0.077	0.1356	0.399	1.78E-09
GIMAP6	7	-0.077	0.1362	0.359	8.32E-08
RAB20	13	0.077	0.1373	0.354	1.30E-07
GADD45B	19	-0.077	0.1377	0.386	6.69E-09
ZNF711	X	0.077	0.1392	-0.396	2.59E-09
TOB1	17	-0.076	0.1419	0.497	1.47E-14
KCNQ1	11	-0.076	0.1427	0.328	1.09E-06
TBL1X	X	0.075	0.1457	0.493	2.44E-14
SLFN12	17	0.075	0.1458	0.367	4.08E-08
SLC16A3	17	0.075	0.1472	0.352	1.50E-07
JUNB	19	0.075	0.1473	0.413	4.08E-10
CYP1B1	2	0.075	0.1482	0.475	2.90E-13
SIGLEC5	19	0.075	0.1493	0.405	1.03E-09
CRYL1	13	-0.075	0.1495	0.475	2.76E-13
SEMA6C	1	-0.075	0.1497	0.378	1.37E-08
LRRC8D	1	-0.075	0.1498	0.438	2.77E-11
PELI1	2	0.075	0.1501	0.319	2.23E-06
GAS7	17	-0.074	0.1522	0.338	5.05E-07
CAPZB	1	-0.074	0.1537	0.415	3.51E-10
CTSA	20	0.073	0.1573	0.366	4.34E-08
MOSPD2	X	-0.073	0.1582	0.368	3.57E-08
SLC7A7	14	0.073	0.1583	0.346	2.44E-07
SMYD3	1	-0.073	0.1585	-0.331	9.01E-07
SH2B3	12	0.073	0.1589	0.331	8.38E-07
PRKCA	17	-0.073	0.1595	0.504	5.11E-15
FZD7	2	0.073	0.1608	-0.329	1.01E-06
RAD51AP1	12	0.073	0.1616	-0.342	3.44E-07
PPARD	6	0.072	0.1648	0.337	5.55E-07
KIF1B	1	-0.072	0.1655	0.351	1.68E-07
STAT6	12	0.072	0.1662	0.415	3.43E-10
EPHX2	8	0.072	0.1663	0.343	3.17E-07
TNFSF13	17	0.071	0.1685	0.501	8.88E-15
ACTN1	14	-0.071	0.1705	0.421	1.75E-10

PIAS1	15	-0.071	0.1705	0.380	1.21E-08
NUSAP1	15	-0.071	0.1737	-0.321	1.99E-06
FRAT2	10	-0.070	0.1748	0.438	2.77E-11
CD93	20	-0.070	0.1763	0.401	1.48E-09
TGFBR2	3	-0.070	0.1775	0.384	8.36E-09
TRAF3IP3	1	0.070	0.1779	0.365	4.78E-08
RASSF2	20	0.070	0.1785	0.394	3.00E-09
DISC1	1	0.070	0.1790	0.673	0
SLC11A1	2	0.069	0.1816	0.358	8.85E-08
OSTF1	9	0.069	0.1852	0.335	6.39E-07
CD48	1	-0.069	0.1856	0.431	6.23E-11
GCH1	14	-0.069	0.1860	0.401	1.46E-09
DDAH1	1	0.068	0.1880	-0.355	1.12E-07
RUFY2	10	0.068	0.1901	0.419	2.24E-10
CDC42BPA	1	0.068	0.1923	-0.357	9.63E-08
CNIH4	1	0.067	0.1947	0.499	1.11E-14
RPS6KA4	11	0.067	0.1984	0.466	8.85E-13
PHC2	1	0.066	0.2004	0.320	2.14E-06
ARL4C	2	-0.066	0.2020	0.373	2.36E-08
AHR	7	0.066	0.2028	0.383	8.85E-09
DOK2	8	-0.066	0.2031	0.457	2.91E-12
CORO1A	16	-0.066	0.2039	0.437	3.00E-11
FCGRT	19	0.065	0.2119	0.481	1.31E-13
AOAH	7	0.064	0.2152	0.327	1.15E-06
STX6	1	0.064	0.2155	0.352	1.50E-07
GRN	17	0.064	0.2178	0.445	1.12E-11
SCRN3	2	0.064	0.2205	0.406	9.07E-10
MAL	2	-0.063	0.2269	0.414	3.73E-10
ITPK1	14	0.062	0.2317	0.327	1.17E-06
ATG7	3	-0.062	0.2329	0.505	4.88E-15
IL6ST	5	0.062	0.2347	0.518	6.66E-16
IL16	15	-0.062	0.2357	0.409	6.70E-10
VIM	10	0.061	0.2362	0.438	2.72E-11
RAB27A	15	-0.061	0.2363	0.357	9.43E-08
TLR2	4	-0.061	0.2377	0.534	0
LILRA6	19	0.061	0.2402	0.317	2.52E-06
PPT1	1	0.061	0.2430	0.517	8.88E-16
PPFIA1	11	0.060	0.2452	0.333	7.39E-07
CKS2	9	-0.060	0.2494	-0.394	2.93E-09
SERPINB8	18	0.059	0.2530	0.426	1.01E-10
MYD88	3	0.059	0.2540	0.440	2.13E-11
CD46	1	-0.059	0.2542	0.345	2.67E-07
SH3BP2	4	0.059	0.2548	0.318	2.47E-06
REEP5	5	-0.059	0.2555	0.380	1.13E-08
PRRG4	11	-0.059	0.2561	0.343	3.21E-07

NCF2	1	0.059	0.2587	0.526	2.22E-16
PLCL1	2	-0.058	0.2613	0.494	2.31E-14
TRIM38	6	0.058	0.2635	0.337	5.24E-07
MCM2	3	0.058	0.2650	-0.359	8.27E-08
UPP1	7	0.058	0.2655	0.498	1.20E-14
ST7	7	0.058	0.2665	-0.343	3.23E-07
GALNS	16	-0.057	0.2733	0.351	1.71E-07
TBC1D4	13	0.057	0.2734	0.377	1.63E-08
APOL3	22	0.057	0.2743	0.418	2.52E-10
NFE2L2	2	0.056	0.2767	0.373	2.36E-08
GNG10	9	0.056	0.2803	0.322	1.79E-06
CAB39	2	0.056	0.2828	0.351	1.69E-07
F5	1	0.056	0.2837	0.580	0
TNFAIP3	6	0.056	0.2841	0.359	8.32E-08
SLC15A3	11	0.056	0.2850	0.445	1.22E-11
OSBPL11	3	0.055	0.2921	0.320	2.05E-06
SLC6A6	3	0.055	0.2925	0.322	1.82E-06
PEA15	1	-0.055	0.2932	0.328	1.12E-06
KDELC1	13	0.054	0.2995	-0.388	5.37E-09
PCYOX1L	5	-0.054	0.3010	0.323	1.58E-06
SYNJ1	21	0.053	0.3040	0.482	1.14E-13
CKLF	16	-0.053	0.3093	0.400	1.58E-09
CLTB	5	-0.053	0.3093	0.318	2.40E-06
PDGFD	11	-0.053	0.3118	-0.406	9.01E-10
SNX27	1	-0.052	0.3128	0.561	0
N4BP1	16	0.052	0.3128	0.435	3.66E-11
FBN1	15	0.052	0.3146	-0.324	1.56E-06
MX2	21	0.052	0.3153	0.573	0
NPTN	15	-0.052	0.3154	0.423	1.51E-10
PIK3CD	1	0.052	0.3192	0.352	1.51E-07
LPXN	11	-0.052	0.3197	0.394	3.14E-09
IRAK4	12	-0.051	0.3222	0.332	8.19E-07
LDLRAP1	1	0.051	0.3245	0.349	2.04E-07
TBC1D2B	15	0.051	0.3266	0.426	1.02E-10
GIT2	12	-0.051	0.3271	0.333	7.24E-07
CRK	17	0.051	0.3277	0.349	1.91E-07
HERC3	4	-0.051	0.3286	0.373	2.22E-08
SLC25A44	1	-0.050	0.3348	0.331	8.60E-07
DEGS1	1	0.050	0.3376	0.331	8.63E-07
FTH1	11	0.050	0.3398	0.442	1.75E-11
CHMP4A	14	0.049	0.3412	0.336	5.89E-07
SNAP23	15	-0.049	0.3417	0.338	4.92E-07
NETO2	16	-0.049	0.3422	0.399	1.78E-09
WDFY3	4	-0.049	0.3449	0.428	8.30E-11
WARS	14	0.049	0.3449	0.415	3.49E-10

STX10	19	0.049	0.3467	0.358	9.24E-08
CAMKK2	12	0.049	0.3481	0.359	8.51E-08
IL13RA1	X	0.049	0.3483	0.412	4.78E-10
ZFYVE16	5	0.048	0.3531	0.341	3.70E-07
SERPINA1	14	0.048	0.3551	0.424	1.35E-10
TIMELESS	12	-0.048	0.3595	-0.323	1.59E-06
DPEP2	16	0.047	0.3614	0.459	2.08E-12
S100A6	1	0.047	0.3615	0.477	2.25E-13
ARHGEF11	1	0.047	0.3653	0.618	0
COG5	7	0.047	0.3664	0.323	1.68E-06
ALDOA	16	-0.047	0.3682	0.335	6.05E-07
MTUS1	8	-0.046	0.3736	0.361	7.04E-08
EVI2B	17	0.046	0.3737	0.378	1.47E-08
CERK	22	0.046	0.3743	0.460	2.03E-12
GCSH	16	-0.046	0.3744	-0.348	2.14E-07
IER3	6	0.046	0.3769	0.456	2.98E-12
GIMAP5	7	-0.046	0.3788	0.409	6.36E-10
GLCE	15	0.045	0.3863	0.334	6.93E-07
AMPD2	1	-0.045	0.3893	0.351	1.65E-07
TMEM43	3	-0.045	0.3908	0.318	2.39E-06
TRIM14	9	0.044	0.3936	0.437	2.93E-11
RNASET2	6	0.044	0.3959	0.424	1.31E-10
PRMT2	21	-0.044	0.3962	0.359	8.14E-08
OAS3	12	0.044	0.3969	0.461	1.62E-12
CNDP2	18	0.044	0.3991	0.351	1.58E-07
DLG5	10	-0.044	0.3992	-0.320	2.07E-06
SIRT7	17	-0.044	0.3994	0.325	1.39E-06
NLRP1	17	0.043	0.4069	0.518	6.66E-16
HEATR3	16	-0.043	0.4131	0.440	2.15E-11
SERPINI1	3	-0.042	0.4135	-0.424	1.23E-10
CFP	X	-0.042	0.4209	0.494	2.20E-14
MYH9	22	0.042	0.4227	0.349	1.91E-07
PEPD	19	0.042	0.4235	0.349	1.92E-07
CHMP7	8	0.041	0.4249	0.326	1.27E-06
ALDH5A1	6	-0.041	0.4267	-0.327	1.17E-06
OSTM1	6	0.041	0.4275	0.339	4.41E-07
ERO1L	14	0.041	0.4283	0.345	2.81E-07
STX16	20	-0.041	0.4285	0.383	8.52E-09
ARL6IP5	3	0.041	0.4310	0.349	2.02E-07
SORL1	11	-0.041	0.4326	0.417	2.69E-10
SLC16A5	17	0.041	0.4328	0.443	1.40E-11
MAN2B1	19	-0.041	0.4331	0.425	1.21E-10
MAN1C1	1	0.041	0.4348	0.534	0
RAD51	15	-0.040	0.4367	-0.408	7.00E-10
PDE3B	11	-0.040	0.4378	0.375	1.93E-08

DTL	1	0.040	0.4381	-0.357	9.61E-08
HTRA2	2	-0.040	0.4384	-0.341	3.83E-07
MAN2C1	15	0.040	0.4401	0.364	5.25E-08
EVI2A	17	0.040	0.4429	0.546	0
PDXK	21	-0.040	0.4433	0.505	4.88E-15
MINA	3	0.040	0.4445	0.439	2.45E-11
GINS2	16	0.040	0.4450	-0.351	1.71E-07
NDST2	10	-0.040	0.4453	0.348	2.04E-07
SEMA4A	1	0.040	0.4463	0.519	6.66E-16
ANKS1A	6	0.039	0.4491	0.338	5.10E-07
LILRB2	19	0.039	0.4506	0.344	3.09E-07
STAT1	2	0.039	0.4525	0.429	7.25E-11
STK10	5	0.039	0.4537	0.354	1.24E-07
NUP62	19	-0.039	0.4567	0.330	9.46E-07
CEBPB	20	0.039	0.4570	0.379	1.24E-08
USP4	3	-0.039	0.4583	0.378	1.38E-08
TRIM36	5	-0.038	0.4622	0.458	2.50E-12
LACTB2	8	-0.038	0.4652	0.320	2.09E-06
PFKFB3	10	-0.038	0.4670	0.323	1.62E-06
SGSH	17	-0.038	0.4671	0.521	4.44E-16
UBE2D1	10	0.038	0.4693	0.398	2.05E-09
SCO2	22	0.037	0.4722	0.475	2.78E-13
NCAPG	4	-0.037	0.4722	-0.319	2.17E-06
SMPD4	2	-0.037	0.4738	0.318	2.49E-06
SQRDL	15	-0.037	0.4744	0.488	4.80E-14
RHOG	11	-0.037	0.4745	0.394	2.93E-09
ZDHHC18	1	0.037	0.4751	0.396	2.52E-09
MYO5A	15	0.037	0.4768	0.348	2.07E-07
SAMHD1	20	0.037	0.4768	0.609	0
CSAD	12	0.037	0.4803	0.324	1.48E-06
GNS	12	-0.037	0.4806	0.382	1.02E-08
CHAF1B	21	-0.037	0.4808	-0.417	2.74E-10
S100A11	1	0.036	0.4861	0.458	2.45E-12
ALDH2	12	0.036	0.4868	0.373	2.20E-08
USP3	15	-0.036	0.4873	0.425	1.11E-10
MX1	21	0.036	0.4875	0.317	2.58E-06
PYGB	20	-0.036	0.4885	0.323	1.62E-06
ZNF200	16	0.036	0.4911	0.338	4.84E-07
ICAM1	19	0.036	0.4915	0.413	4.24E-10
SOCS3	17	-0.036	0.4922	0.463	1.35E-12
KLHL23	2	0.036	0.4938	-0.381	1.06E-08
LCP2	5	0.035	0.4945	0.401	1.51E-09
CTSS	1	0.035	0.4955	0.423	1.48E-10
CPD	17	-0.035	0.4956	0.344	2.89E-07
MTF1	1	0.035	0.4956	0.356	1.06E-07

KIAA1467	12	0.035	0.4962	-0.342	3.48E-07
WAS	X	0.035	0.4993	0.461	1.76E-12
HIPK3	11	-0.035	0.4994	0.399	1.90E-09
LEPROTL1	8	-0.035	0.5047	0.360	7.67E-08
CRIP1	14	0.035	0.5057	0.378	1.41E-08
ITM2B	13	-0.034	0.5066	0.399	1.90E-09
TNFSF10	3	0.034	0.5084	0.493	2.46E-14
MKL1	22	0.034	0.5088	0.408	7.42E-10
PHACTR2	6	0.034	0.5149	0.361	7.01E-08
CASP1	11	-0.034	0.5170	0.557	0
OAS2	12	0.034	0.5180	0.412	4.69E-10
TLR1	4	0.034	0.5181	0.448	8.07E-12
ZCCHC14	16	-0.033	0.5192	0.340	4.27E-07
HIST1H3D	6	0.033	0.5223	-0.347	2.37E-07
PLP2	X	0.033	0.5256	0.395	2.62E-09
TMEM30A	6	-0.033	0.5260	0.331	8.51E-07
HEXB	5	-0.033	0.5271	0.455	3.56E-12
KIAA1324	1	-0.033	0.5277	0.323	1.66E-06
GLIPR1	12	0.032	0.5338	0.351	1.63E-07
WDR7	18	0.032	0.5354	0.320	2.14E-06
DMXL2	15	-0.032	0.5383	0.407	8.09E-10
MPPE1	18	-0.032	0.5408	0.329	9.91E-07
IMPDH1	7	0.032	0.5413	0.334	7.08E-07
SEC23B	20	-0.032	0.5415	0.364	5.36E-08
ACVR2A	2	0.032	0.5418	0.437	2.85E-11
MNT	17	0.032	0.5422	0.331	8.83E-07
PDLIM5	4	-0.032	0.5429	0.362	6.00E-08
DGKA	12	0.032	0.5435	0.395	2.83E-09
PAK1	11	0.031	0.5490	0.337	5.54E-07
SLC17A5	6	0.031	0.5493	0.405	9.98E-10
VNN2	6	-0.031	0.5510	0.424	1.36E-10
GSTK1	7	0.031	0.5526	0.379	1.32E-08
POLR3G	5	-0.031	0.5532	-0.386	6.39E-09
EXT1	8	-0.031	0.5558	0.408	7.26E-10
PTPRE	10	-0.031	0.5567	0.420	1.93E-10
AGTPBP1	9	0.030	0.5584	0.391	4.25E-09
ANXA5	4	0.030	0.5622	0.481	1.34E-13
IL10RB	21	0.030	0.5624	0.389	5.06E-09
SLC2A3	12	0.030	0.5630	0.360	7.57E-08
ABCB1	7	-0.030	0.5666	-0.394	2.89E-09
STOML2	9	0.030	0.5688	-0.346	2.42E-07
FHIT	3	-0.030	0.5696	0.346	2.50E-07
TM9SF4	20	-0.030	0.5700	0.390	4.59E-09
DPYD	1	0.029	0.5713	0.434	4.11E-11
WWP1	8	-0.029	0.5744	0.381	1.06E-08

MYL6B	12	-0.029	0.5744	-0.429	7.74E-11
ATP1A1	1	-0.029	0.5786	0.457	2.79E-12
POLE2	14	-0.028	0.5843	-0.331	8.73E-07
MEGF9	9	0.028	0.5849	0.431	5.70E-11
RBMS1	2	-0.028	0.5860	0.440	2.12E-11
WASF1	6	0.028	0.5879	-0.388	5.24E-09
DNASE1L1	X	0.028	0.5903	0.321	1.96E-06
TPP1	11	-0.028	0.5931	0.398	1.94E-09
GSTA4	6	0.028	0.5943	-0.355	1.14E-07
STK38L	12	0.028	0.5956	0.324	1.53E-06
PSAP	10	-0.027	0.5984	0.323	1.66E-06
NDRG1	8	-0.027	0.5991	0.428	8.57E-11
ATP6V1B2	8	0.027	0.5999	0.440	2.04E-11
DPP4	2	0.027	0.6065	0.322	1.71E-06
RNH1	11	0.027	0.6072	0.425	1.13E-10
TOR1AIP1	1	-0.027	0.6088	0.434	4.41E-11
GPSM3	6	-0.026	0.6140	0.473	3.74E-13
TNFSF12	17	-0.026	0.6142	0.407	7.69E-10
ITGB2	21	-0.026	0.6168	0.477	2.10E-13
FLNA	X	0.026	0.6199	0.324	1.47E-06
CSF1R	5	0.025	0.6285	0.462	1.58E-12
CDK6	7	-0.025	0.6293	-0.358	8.83E-08
PILRA	7	-0.025	0.6321	0.423	1.37E-10
NSF	17	0.025	0.6324	0.374	2.08E-08
ST6GALNAC2	17	-0.025	0.6333	0.383	8.79E-09
LRCH4	7	-0.025	0.6338	0.373	2.22E-08
PTAFR	1	0.025	0.6350	0.476	2.57E-13
GPR65	14	-0.025	0.6364	0.336	5.57E-07
RNF44	5	-0.024	0.6394	0.319	2.16E-06
PIK3R3	1	0.024	0.6398	-0.361	6.92E-08
PIK3CB	3	-0.024	0.6450	0.323	1.69E-06
FBN2	5	-0.024	0.6454	0.330	9.39E-07
KLHL22	22	0.024	0.6463	0.325	1.44E-06
LRPAP1	4	0.024	0.6469	0.343	3.30E-07
SLC9A6	X	-0.024	0.6475	0.328	1.11E-06
ID2	2	-0.023	0.6582	0.324	1.57E-06
FGD6	12	0.023	0.6589	0.343	3.19E-07
GNAI2	3	-0.023	0.6591	0.361	6.74E-08
ARF5	7	-0.023	0.6623	0.351	1.71E-07
TBC1D8	2	0.022	0.6682	0.361	6.96E-08
RGL1	1	-0.022	0.6692	0.699	0
STX3	11	0.022	0.6695	0.362	6.24E-08
CLEC4A	12	0.022	0.6728	0.503	6.22E-15
CYBA	16	-0.022	0.6761	0.334	6.59E-07
HLA-B	6	0.022	0.6768	0.338	4.96E-07

HMMR	5	0.022	0.6772	-0.322	1.74E-06
NAGA	22	0.021	0.6821	0.525	2.22E-16
EHD4	15	0.021	0.6837	0.507	3.55E-15
NOD2	16	0.021	0.6846	0.648	0
SLC7A6	16	0.021	0.6848	0.348	2.14E-07
DOCK4	7	0.021	0.6877	0.374	2.11E-08
DBN1	5	-0.021	0.6879	-0.326	1.27E-06
SETX	9	0.021	0.6880	0.330	9.10E-07
ENTPD6	20	-0.021	0.6897	0.330	9.75E-07
MYH10	17	-0.021	0.6907	-0.368	3.46E-08
LILRB1	19	0.021	0.6910	0.425	1.09E-10
NPC2	14	0.021	0.6918	0.319	2.32E-06
SMARCD3	7	-0.021	0.6929	0.512	1.78E-15
TIAM1	21	-0.021	0.6930	0.661	0
C1GALT1C1	X	0.021	0.6930	0.317	2.70E-06
LRP1	12	0.020	0.6953	0.486	6.62E-14
ACAA1	3	0.020	0.6971	0.351	1.70E-07
MTMR11	1	0.020	0.6975	0.546	0
DYNLT1	6	-0.020	0.6980	0.354	1.21E-07
TGOLN2	2	-0.020	0.6983	0.433	4.83E-11
FYB	5	-0.020	0.6983	0.511	1.78E-15
TAPBPL	12	0.020	0.7003	0.322	1.74E-06
CBX6	22	0.020	0.7020	0.322	1.75E-06
PSEN1	14	-0.020	0.7038	0.364	5.45E-08
CTBS	1	-0.020	0.7043	0.409	6.40E-10
TFDP2	3	-0.020	0.7043	-0.424	1.32E-10
ARNTL	11	0.020	0.7070	0.415	3.46E-10
GIMAP4	7	0.019	0.7102	0.536	0
ENTPD1	10	0.019	0.7106	0.419	2.35E-10
PMM1	22	-0.019	0.7119	0.385	7.17E-09
AGER	6	-0.019	0.7149	0.318	2.44E-06
GLRX	5	-0.019	0.7171	0.429	7.13E-11
ZNF217	20	-0.019	0.7186	0.338	5.09E-07
RPS6KA3	X	-0.019	0.7190	0.401	1.53E-09
CYP27A1	2	0.019	0.7193	0.517	8.88E-16
CREB5	7	0.019	0.7211	0.494	2.22E-14
AIP	11	-0.018	0.7232	0.319	2.27E-06
ZYX	7	-0.018	0.7233	0.461	1.75E-12
CYLD	16	0.018	0.7235	0.322	1.81E-06
CLN3	16	0.018	0.7268	0.359	8.34E-08
IL10RA	11	0.018	0.7286	0.441	1.91E-11
RPS6KA1	1	0.018	0.7309	0.364	4.98E-08
ANXA1	9	-0.018	0.7320	0.431	5.98E-11
CTSH	15	-0.018	0.7340	0.343	3.22E-07
TCF4	18	-0.018	0.7351	-0.324	1.53E-06

ATF6	1	0.018	0.7356	0.326	1.31E-06
PGS1	17	-0.017	0.7380	0.365	4.60E-08
LAMP3	3	-0.017	0.7404	0.407	7.88E-10
KIAA0125	14	0.017	0.7404	-0.386	6.64E-09
C1orf21	1	0.017	0.7425	-0.319	2.26E-06
IL27RA	19	-0.017	0.7428	0.400	1.67E-09
SASH1	6	-0.017	0.7437	0.434	4.23E-11
DLEU1	13	0.017	0.7452	-0.330	9.47E-07
PXN	12	0.017	0.7456	0.437	2.87E-11
LGALS1	22	0.017	0.7457	0.349	1.94E-07
COL5A1	9	0.017	0.7475	-0.328	1.11E-06
MAP2K1	15	-0.017	0.7476	0.415	3.58E-10
BLM	15	0.016	0.7509	-0.357	9.65E-08
RASA4	7	0.016	0.7515	0.443	1.51E-11
BSPRY	9	-0.016	0.7521	-0.359	7.92E-08
EDEM3	1	-0.016	0.7521	0.413	4.38E-10
TYK2	19	-0.016	0.7523	0.403	1.21E-09
TRPS1	8	-0.016	0.7529	0.331	8.93E-07
TSPAN14	10	0.016	0.7535	0.361	6.70E-08
GDAP2	1	0.016	0.7562	0.360	7.22E-08
TNFRSF10B	8	-0.016	0.7565	0.431	5.62E-11
TRADD	16	0.016	0.7568	0.351	1.69E-07
RCBTB2	13	-0.016	0.7610	0.492	2.73E-14
HMHA1	19	-0.016	0.7639	0.369	3.40E-08
ITGAL	16	0.015	0.7670	0.367	3.81E-08
CKAP4	12	0.015	0.7789	0.453	4.37E-12
TLE4	9	0.015	0.7792	0.363	5.62E-08
PLCB2	15	-0.014	0.7812	0.410	5.75E-10
KIAA0319L	1	-0.014	0.7816	0.400	1.68E-09
RNF13	3	0.014	0.7872	0.329	1.00E-06
MAP3K1	5	0.014	0.7883	0.363	5.72E-08
ACVR1B	12	0.014	0.7885	0.335	6.22E-07
TUBD1	17	0.014	0.7893	0.332	8.30E-07
TAP1	6	0.014	0.7897	0.324	1.51E-06
ANXA2	15	0.014	0.7907	0.365	4.65E-08
GLB1	3	-0.014	0.7915	0.520	4.44E-16
GABBR1	6	0.014	0.7927	0.443	1.48E-11
LRRFIP1	2	0.014	0.7949	0.378	1.37E-08
ATP6V0E1	5	-0.013	0.7950	0.354	1.24E-07
UROS	10	-0.013	0.7967	-0.334	6.72E-07
KLF4	9	0.013	0.7976	0.340	4.06E-07
ARHGAP26	5	-0.013	0.7997	0.358	9.17E-08
TIMP1	X	-0.013	0.8003	0.441	1.91E-11
GGA3	17	0.013	0.8006	0.320	2.15E-06
PAWR	12	0.013	0.8058	-0.398	1.95E-09

SPATS2	12	0.012	0.8102	-0.373	2.35E-08
ARRB2	17	0.012	0.8105	0.578	0
YWHAB	20	-0.012	0.8106	0.400	1.60E-09
LAPTM5	1	0.012	0.8106	0.344	2.94E-07
MTO1	6	0.012	0.8118	0.332	7.86E-07
YPEL1	22	-0.012	0.8128	-0.430	6.92E-11
MTMR2	11	-0.012	0.8138	-0.344	2.88E-07
AZI2	3	-0.012	0.8165	0.363	5.47E-08
MTMR14	3	0.012	0.8175	0.343	3.27E-07
WDR1	4	-0.011	0.8272	0.374	2.15E-08
TSPO	22	-0.011	0.8290	0.474	3.27E-13
LAMC1	1	-0.011	0.8311	-0.354	1.30E-07
PACSIN2	22	-0.011	0.8346	0.361	7.10E-08
WEE1	11	0.010	0.8409	-0.509	2.83E-15
TPK1	7	-0.010	0.8431	0.538	0
WAPAL	10	-0.010	0.8434	0.353	1.34E-07
IRS2	13	0.010	0.8446	0.323	1.63E-06
SEL1L	14	-0.010	0.8486	0.334	6.61E-07
AKAP13	15	0.010	0.8519	0.383	9.03E-09
HIST1H1C	6	0.010	0.8521	-0.359	8.08E-08
SCML1	X	-0.009	0.8552	0.459	2.21E-12
CRTAP	3	0.009	0.8552	0.321	1.97E-06
MTHFS	15	0.009	0.8561	0.324	1.55E-06
M6PR	12	0.009	0.8583	0.391	3.91E-09
VPS54	2	0.009	0.8602	0.323	1.65E-06
SULT1A4	16	-0.009	0.8616	0.333	7.63E-07
ADI1	2	-0.009	0.8621	0.352	1.55E-07
MAK	6	0.009	0.8623	0.343	3.17E-07
OS9	12	-0.009	0.8645	0.411	5.44E-10
TREX1	3	-0.009	0.8647	0.337	5.50E-07
MAP3K3	17	0.009	0.8657	0.523	4.44E-16
UBL3	13	-0.009	0.8672	0.410	5.94E-10
PHF7	3	-0.009	0.8684	-0.374	2.17E-08
TGFBI	5	-0.009	0.8688	0.456	2.95E-12
HSPBAP1	3	-0.008	0.8702	0.415	3.43E-10
MIPEP	13	0.008	0.8710	-0.335	6.35E-07
CD28	2	0.008	0.8730	0.335	6.52E-07
VANGL1	1	0.008	0.8733	-0.322	1.78E-06
NAIP	5	0.008	0.8740	0.492	2.84E-14
FRAT1	10	-0.008	0.8804	0.422	1.59E-10
NXF1	11	-0.008	0.8845	0.342	3.66E-07
VASH1	14	0.007	0.8858	0.423	1.51E-10
FKBP15	9	-0.007	0.8868	0.495	1.91E-14
MTCH1	6	0.007	0.8885	0.349	1.88E-07
CECR5	22	0.007	0.8895	0.421	1.76E-10

ARHGEF10L	1	0.007	0.8902	0.514	1.33E-15
PLAUR	19	-0.007	0.8930	0.346	2.54E-07
LAP3	4	-0.007	0.8933	0.363	5.47E-08
SAT1	X	0.007	0.8938	0.395	2.60E-09
NUP214	9	0.007	0.8951	0.498	1.29E-14
ARPC5	1	-0.007	0.8954	0.349	1.98E-07
FAM45B	X	-0.007	0.8968	0.342	3.45E-07
PSMC3IP	17	-0.007	0.8970	0.364	5.20E-08
PGK1	X	-0.007	0.8984	0.401	1.51E-09
IFNGR1	6	0.007	0.8993	0.342	3.56E-07
IFNAR2	21	-0.007	0.9000	0.434	4.11E-11
SERPINB2	18	0.007	0.9001	0.362	6.45E-08
APOBEC3A	22	0.006	0.9005	0.344	2.94E-07
AAK1	2	-0.006	0.9014	0.371	2.78E-08
PLK4	4	0.006	0.9020	-0.347	2.40E-07
MTMR6	13	-0.006	0.9027	0.384	7.95E-09
DHFR	5	0.006	0.9043	-0.331	8.78E-07
CSTB	21	0.006	0.9116	0.438	2.80E-11
UBQLN2	X	0.006	0.9131	0.350	1.80E-07
HMOX1	22	-0.006	0.9143	0.357	9.61E-08
OCEL1	19	-0.005	0.9157	0.358	8.60E-08
DHX16	6	-0.005	0.9199	0.323	1.65E-06
RNASEL	1	0.005	0.9216	0.479	1.58E-13
BTN2A1	6	0.005	0.9263	0.329	1.06E-06
ALDH3A2	17	0.005	0.9266	0.492	2.89E-14
NFKB1	4	0.005	0.9280	0.425	1.17E-10
CEBPD	8	0.005	0.9300	0.384	7.72E-09
NPEPL1	20	0.005	0.9305	0.323	1.60E-06
HBEGF	5	-0.004	0.9317	0.499	1.11E-14
SORBS1	10	-0.004	0.9355	-0.327	1.21E-06
ELOVL6	4	-0.004	0.9366	-0.358	9.01E-08
CORO2A	9	-0.004	0.9381	0.406	8.49E-10
CNN2	19	0.004	0.9392	0.325	1.36E-06
CAP1	1	-0.004	0.9398	0.375	1.94E-08
MGAT1	5	-0.004	0.9404	0.472	4.26E-13
DECR1	8	-0.004	0.9416	0.318	2.39E-06
TFCP2	12	0.004	0.9426	0.336	5.85E-07
SULT1A1	16	0.004	0.9439	0.429	7.39E-11
DHRS4	14	-0.004	0.9461	0.318	2.36E-06
ATRN	20	-0.003	0.9467	0.343	3.19E-07
INPP4A	2	-0.003	0.9499	0.371	2.63E-08
SLC35A1	6	-0.003	0.9503	0.420	1.90E-10
CAMTA2	17	0.003	0.9519	0.455	3.53E-12
SERINC5	5	0.003	0.9530	0.503	6.66E-15
C5AR1	19	0.003	0.9532	0.345	2.81E-07

DSG2	18	0.003	0.9534	-0.334	6.74E-07
HCLS1	3	0.003	0.9548	0.370	3.02E-08
NAGK	2	-0.003	0.9550	0.494	2.31E-14
VAMP3	1	-0.003	0.9552	0.387	6.22E-09
CST3	20	0.003	0.9558	0.501	8.44E-15
VPS45	1	0.003	0.9573	0.341	3.87E-07
RETSAT	2	-0.003	0.9574	0.320	2.12E-06
SNX1	15	0.003	0.9587	0.397	2.15E-09
PRNP	20	-0.002	0.9625	0.344	2.91E-07
ERP29	12	0.002	0.9702	0.325	1.37E-06
SFXN3	10	0.002	0.9729	0.324	1.53E-06
WDHD1	14	0.002	0.9738	-0.375	1.95E-08
NRBF2	10	0.002	0.9739	0.395	2.64E-09
RRAS2	11	0.002	0.9769	-0.375	1.87E-08
LILRA1	19	0.001	0.9777	0.396	2.47E-09
PADI2	1	-0.001	0.9778	0.400	1.63E-09
TMEM127	2	-0.001	0.9786	0.340	4.09E-07
KLHL2	4	0.001	0.9828	0.329	1.04E-06
COTL1	16	-0.001	0.9848	0.454	4.23E-12
DAZAP2	12	0.001	0.9878	0.358	8.84E-08
CCDC109B	4	-0.001	0.9887	0.352	1.50E-07
ULK2	17	0.000	0.9943	0.424	1.25E-10
VASP	19	0.000	0.9943	0.338	4.92E-07
ALDH3B1	11	0.000	0.9945	0.343	3.35E-07
ACVR1	2	0.000	0.9951	0.502	7.11E-15
HTATIP2	11	0.000	0.9958	0.321	1.91E-06
HCK	20	0.000	0.9962	0.335	6.19E-07
PHF20L1	8	0.000	0.9965	0.333	7.69E-07
S100A10	1	0.000	0.9974	0.561	0
SLC31A2	9	0.000	0.9978	0.456	3.27E-12
RASGRP3	2	0.000	0.9997	-0.322	1.84E-06
VIPR1	3	NA	NA	0.736	0
CD163	12	NA	NA	0.591	0
PCSK5	9	NA	NA	0.570	0
RTN1	14	NA	NA	0.556	0
CACNA2D3	3	NA	NA	0.555	0
CRISPLD2	16	NA	NA	0.544	0
FBP1	9	NA	NA	0.530	0
HNMT	2	NA	NA	0.520	4.44E-16
ZNF467	7	NA	NA	0.519	4.44E-16
ASGR2	17	NA	NA	0.519	6.66E-16
TLR5	1	NA	NA	0.518	6.66E-16
TREM1	6	NA	NA	0.518	8.88E-16
TMEM176A	7	NA	NA	0.503	6.22E-15
SIGLEC1	20	NA	NA	0.500	9.77E-15

LGALS2	22	NA	NA	0.496	1.69E-14
ADAMTSL4	1	NA	NA	0.494	2.26E-14
SECTM1	17	NA	NA	0.493	2.53E-14
SULT1B1	4	NA	NA	0.493	2.53E-14
VSIG4	X	NA	NA	0.491	3.22E-14
VENTX	10	NA	NA	0.491	3.29E-14
FCN1	9	NA	NA	0.491	3.31E-14
MS4A6A	11	NA	NA	0.489	4.55E-14
FCGR1B	1	NA	NA	0.487	6.15E-14
KBTBD11	8	NA	NA	0.474	3.40E-13
FCGR1A	1	NA	NA	0.473	3.71E-13
CD14	5	NA	NA	0.469	6.09E-13
CCR2	3	NA	NA	0.467	8.00E-13
CSF2RA	X	NA	NA	0.467	8.23E-13
TMEM176B	7	NA	NA	0.466	9.20E-13
MRC1	10	NA	NA	0.461	1.73E-12
MAPK10	4	NA	NA	0.453	4.34E-12
STEAP4	7	NA	NA	0.453	4.40E-12
P2RY13	3	NA	NA	0.452	5.20E-12
CLEC4E	12	NA	NA	0.452	5.36E-12
FPR1	19	NA	NA	0.439	2.24E-11
SEZ6L	22	NA	NA	0.433	4.46E-11
IL1RN	2	NA	NA	0.433	4.60E-11
MEFV	16	NA	NA	0.433	4.67E-11
TLR8	X	NA	NA	0.432	5.37E-11
PPARGC1A	4	NA	NA	0.428	7.94E-11
RNASE4	14	NA	NA	0.427	9.46E-11
CSTA	3	NA	NA	0.419	2.31E-10
IGSF6	16	NA	NA	0.415	3.36E-10
VNN3	6	NA	NA	0.407	8.35E-10
CSF3R	1	NA	NA	0.406	8.54E-10
DSG1	18	NA	NA	0.404	1.13E-09
CLEC7A	12	NA	NA	0.388	5.59E-09
THBD	20	NA	NA	0.382	9.82E-09
CLEC10A	17	NA	NA	0.381	1.10E-08
SMPDL3A	6	NA	NA	0.372	2.43E-08
FCAR	19	NA	NA	0.372	2.50E-08
CACNA1I	22	NA	NA	0.368	3.51E-08
TGFA	2	NA	NA	0.362	6.11E-08
EPB41L3	18	NA	NA	0.359	8.34E-08
CPVL	7	NA	NA	0.358	8.56E-08
CALCA	11	NA	NA	0.358	8.74E-08
MAFB	20	NA	NA	0.356	1.11E-07
STAB1	3	NA	NA	0.354	1.27E-07
ICOS	2	NA	NA	0.353	1.37E-07

CD5	11	NA	NA	0.346	2.58E-07
GNAQ	9	NA	NA	0.345	2.69E-07
SLA	8	NA	NA	0.344	3.00E-07
CDA	1	NA	NA	0.344	3.01E-07
HAL	12	NA	NA	0.339	4.41E-07
EGLN2	19	NA	NA	0.337	5.49E-07
CLEC1A	12	NA	NA	0.336	5.57E-07
HK3	5	NA	NA	0.334	6.82E-07
LILRA5	19	NA	NA	0.333	7.62E-07
S100A12	1	NA	NA	0.332	7.97E-07
NBPF14	1	NA	NA	0.328	1.13E-06
RIN2	20	NA	NA	0.326	1.30E-06
BCL6	3	NA	NA	0.324	1.49E-06
NBPF10	1	NA	NA	0.321	1.90E-06
CEBPA	19	NA	NA	0.321	1.97E-06
SLC8A1	2	NA	NA	0.317	2.67E-06
CFH	1	NA	NA	-0.317	2.61E-06
MPDZ	9	NA	NA	-0.318	2.51E-06
SPTA1	1	NA	NA	-0.318	2.38E-06
CRYGD	2	NA	NA	-0.319	2.20E-06
CRHBP	5	NA	NA	-0.321	1.93E-06
HIST1H2BE	6	NA	NA	-0.323	1.63E-06
GSTM5	1	NA	NA	-0.324	1.50E-06
MPPED2	11	NA	NA	-0.324	1.48E-06
MYOZ3	5	NA	NA	-0.325	1.46E-06
HMGA2	12	NA	NA	-0.325	1.45E-06
HIST1H2BF	6	NA	NA	-0.325	1.38E-06
NPR3	5	NA	NA	-0.329	9.83E-07
HIST1H2BI	6	NA	NA	-0.330	9.52E-07
TEK	9	NA	NA	-0.331	8.60E-07
IGLL1	22	NA	NA	-0.331	8.59E-07
HIST1H2BH	6	NA	NA	-0.336	6.03E-07
COBLL1	2	NA	NA	-0.336	5.86E-07
ENAH	1	NA	NA	-0.336	5.66E-07
ADAMTS3	4	NA	NA	-0.338	5.01E-07
KHDRBS2	6	NA	NA	-0.339	4.62E-07
KLF1	19	NA	NA	-0.339	4.38E-07
AGTR1	3	NA	NA	-0.349	1.97E-07
HIST1H4C	6	NA	NA	-0.352	1.48E-07
HIST1H3C	6	NA	NA	-0.355	1.20E-07
HOXA10	7	NA	NA	-0.358	9.16E-08
AKR1C2	10	NA	NA	-0.359	8.42E-08
MYT1L	2	NA	NA	-0.360	7.55E-08
FAT4	4	NA	NA	-0.360	7.26E-08
JAKMIP2	5	NA	NA	-0.361	6.68E-08

NUDT11	X	NA	NA	-0.363	5.90E-08
CYTL1	4	NA	NA	-0.366	4.35E-08
TFPI	2	NA	NA	-0.371	2.66E-08
AKR1C3	10	NA	NA	-0.398	2.01E-09
TSPAN13	7	NA	NA	-0.432	5.59E-11

Supplementary Table 4.2. eQTLs for genes co-expressed with *IL6R*.

*Proxy SNPs were chosen based on an $r^2 > 0.8$. Abbreviations: BP, base pair; Chr, chromosome; SNP, single nucleotide polymorphism.

No	SNP	Chr	bp	Gene regulated by a risk SNP	Proxy SNP*	*P*-value	Sample	Study
1	rs9611485	22	41060886	EP300	rs9611462	0	Lung	[194]
	rs9611485	22	41060886	PMM1	rs9611485	4.67E-06	LCLs	[181]
2	rs324011	12	57108399	STAT6	rs12368672	9.81E-198	Whole-Blood	[195]
3	rs8003392	14	76672868	VASH1	rs10873292	9.81E-198	Whole-Blood	[195]
4	rs4911214	20	32299661	TM9SF4	rs6141641	3.03E-184	Whole-Blood	[195]
5	rs4778892	15	81312048	IL16	rs3726	1.57E-183	Whole-Blood	[195]
6	rs10197862	2	102350089	IL18RAP	rs10197862	2.82E-137	Whole-Blood	[195]
	rs10197862	2	102350089	IL18R1	rs950881	0.0003	Whole-Blood	[195]
7	rs731945	19	18456229	ELL	rs731945	1.27E-135	Whole-Blood	[195]
8	rs2267076	22	24434627	UPB1	rs1008931	4.45E-98	Whole-Blood	[195]
9	rs785472	1	46052616	PIK3R3	rs1707321	3.04E-62	Whole-Blood	[197]
10	rs4833095	4	38798089	TLR1	rs12233670	2.80E-57	Whole-Blood	[197]
11	rs7223589	17	31401266	EVI2A	rs10512434	1.32E-56	Whole-Blood	[195]
	rs7223589	17	31401266	EVI2B	rs2525570	2.15E-06	Whole-Blood	[195]
12	rs11602465	11	9943274	ADM	rs12421179	5.90E-56	Whole-Blood	[195]
13	rs13020280	2	223941421	WDFY1	rs6734908	1.83E-43	Whole-Blood	[195]
14	rs10845506	12	12282676	LRP6	rs1071994	2.51E-40	Whole-Blood	[197]
15	rs9289837	3	151388204	MED12L	rs13327359	2.42E-37	Whole-Blood	[195]
	rs9289837	3	151388204	P2RY13	rs7637803	2.01E-14	Whole-Blood	[195]
16	rs6790914	3	49168885	USP4	rs10865954	3.57E-33	Whole-Blood	[195]
	rs6790914	3	49168885	NDUFAF3	rs10865954	0.0034	Whole-Blood	[195]
17	rs7155696	14	35027548	SRP54	rs12890307	1.98E-27	Whole-Blood	[195]
18	rs12502586	4	15724941	FBXL5	rs12502586	6.06E-19	Whole-Blood	[195]
19	rs162328	2	38091546	CYP1B1	rs4670814	6.33E-18	Whole-Blood	[195]
20	rs17061789	3	60014239	FHIT	rs17061789	8.06E-18	Whole-Blood	[195]
21	rs7234644	18	56770128	TXNL1	rs7234644	9.19E-17	Whole-Blood	[195]
22	rs13212921	6	27237643	BTN2A1	rs13217285	1.72E-15	LCLs	[181]
23	rs11265424	1	160530060	SLAMF1	rs6670721	2.96E-15	Whole-Blood	[195]
24	rs17710743	19	41569079	CEACAM21	rs16975338	4.76E-14	Whole-Blood	[195]

25	rs56872707	5	80898586	DHFR	rs13159448	1.42E-13	LCLs	[181]
26	rs10101062	8	123112091	ZHX2	rs7012815	1.78E-13	Whole-Blood	[195]
27	rs4942758	13	48213854	ITM2B	rs551034	2.18E-13	Whole-Blood	[195]
28	rs6949090	7	67432703	STAG3L4	rs10233061	2.99E-12	PBMCs	[193]
29	rs2357792	16	50734311	NOD2	rs7342715	3.45E-11	PBMCs	[193]
30	rs7185536	16	67958261	SLC7A6	rs1107767	4.94E-11	PBMCs	[193]
	rs7185536	16	67958261	PSMB10	rs1133090	3.35E-07	Whole-Blood	[197]
31	rs11000805	10	73903593	NDST2	rs17741873	2.68E-10	Whole-Blood	[195]
32	rs1464510	3	188394766	BCL6	rs9864529	5.22E-10	PBMCs	[193]
33	rs12757280	1	150753573	CTSS	rs55972172	1.35E-09	LCLs	[181]
34	rs9890550	17	75018845	SLC16A5	rs12449492	7.19E-09	Whole-Blood	[195]
35	rs28631821	14	55379269	DLGAP5	rs7147136	1.02E-08	LCLs	[181]
36	rs2419618	10	112316082	ACSL5	rs2419618	1.28E-08	LCLs	[181]
37	rs931702	3	113215905	SIDT1	rs931702	3.10E-08	LCLs	[181]
38	rs13416555	2	8301605	ID2	rs891058	6.05E-08	PBMCs	[193]
39	rs9630721	17	6947049	ASGR2	rs9630722	6.08E-08	Whole-Blood	[195]
40	rs1757460	15	41498262	NUSAP1	rs1757457	8.65E-08	LCLs	[181]
41	rs9298557	8	56981127	IMPAD1	rs9298557	1.03E-07	Lung	[194]
42	rs3827279	22	17115039	IL17RA	rs3827278	3.33E-07	Lung	[194]
43	rs10501420	11	74784992	RNF169	rs17133608	3.49E-07	PBMCs	[193]
44	rs11167763	5	142055901	NDFIP1	rs6884380	5.45E-07	LCLs	[181]
45	rs4653051	1	33333327	PHC2	rs4653015	1.05E-06	Whole-Blood	[197]
46	rs12048743	1	205145745	DSTYK	rs1572993	1.15E-06	Monocytes	[189]
47	rs7946115	11	65598721	FRMD8	rs10791820	1.61E-06	LCLs	[181]
48	rs12709916	19	49817730	PTOV1	rs12709916	1.96E-06	LCLs	[181]
49	rs4725618	7	143425331	ZYX	rs6951852	2.43E-06	Whole-Blood	[195]
50	rs758206	7	140204661	TBXAS1	rs6956540	2.70E-06	Whole-Blood	[195]
51	rs2797414	1	113752816	RSBN1	rs6537798	3.09E-06	Whole-Blood	[195]
52	rs2071595	6	31539285	HLA-B	rs3219184	3.98E-06	LCLs	[181]
53	rs25987	5	14712600	FAM105A	rs31926	5.06E-06	Lung	[194]
54	rs6511788	19	12369223	MAN2B1	rs10411986	5.22E-06	LCLs	[181]
55	rs2453570	17	19474180	ALDH3A2	rs2428584	6.11E-06	Whole-Blood	[195]
56	rs10245342	7	64627050	ZNF736	rs4313046	6.23E-06	LCLs	[181]
57	rs1163073	10	103263177	SH3PXD2A	rs7911488	1.33E-05	Whole-Blood	[195]
58	rs10431490	12	51931787	SMAGP	rs10783481	2.15E-05	Monocytes	[189]
59	rs2033466	5	151701814	SPARC	rs2033466	2.28E-05	Whole-Blood	[195]
60	rs2167524	18	54644208	MBD2	rs1077553	5.15E-05	Lung	[194]
61	rs2435206	17	45980745	NSF	rs2435211	9.38E-05	LCLS	[190]
62	rs7039317	9	34964538	STOML2	rs10972275	9.58E-05	Whole-Blood	[195]
63	rs540178	9	117547445	TLR4	rs540178	0.0002	Whole-Blood	[195]
64	rs13268953	8	28197719	KIF13B	rs13268953	0.0002	Monocytes	[189]
65	rs9989026	12	7105855	CD163	rs6487290	0.0002	Monocytes	[189]
66	rs13256893	8	89664751	RIPK2	rs13256893	0.0003	Whole-Blood	[195]
67	rs34097828	14	94321337	SERPINA1	rs3748320	0.0003	Whole-Blood	[195]
68	rs2071390	16	30070046	CORO1A	rs2071390	0.0003	Whole-Blood	[195]
69	rs2251083	10	133461697	ECHS1	rs2255518	0.0003	Whole-Blood	[195]
70	rs3918188	7	151005693	TMEM176A	rs3918188	0.0004	Whole-Blood	[195]
71	rs7596735	2	44105567	PREPL	rs7596735	0.0004	Whole-Blood	[195]
72	rs2391391	1	94986370	CNN3	rs2391391	0.0004	B-cells	[189]

73	rs6792409	3	69465422	FRMD4B	rs17432953	0.0004	Whole-Blood	[195]
74	rs4687721	3	51564238	PPM1M	rs11719995	0.0005	Monocytes	[189]
75	rs1501534	1	37221817	MTF1	rs1566599	0.0006	B-cells	[189]
76	rs35259724	12	113124090	OAS3	rs17643821	0.0006	B-cells	[189]
77	rs2932967	4	23813502	PPARGC1A	rs2932965	0.0006	Whole-Blood	[195]
78	rs4944804	11	72809485	ARAP1	rs663015	0.0009	Whole-Blood	[195]
79	rs1253118	14	59497301	RTN1	rs956901	0.0016	Whole-Blood	[195]
80	rs1288354	1	53134678	C1orf123	rs1288354	0.0017	Whole-Blood	[195]
81	rs6544629	2	43142628	ZFP36L2	rs12105183	0.0022	Whole-Blood	[195]
82	rs17125240	8	17751387	MTUS1	rs3862101	0.0026	Whole-Blood	[195]
83	rs6134069	20	10882886	JAG1	rs6134067	0.0027	Whole-Blood	[195]
84	rs7246816	19	15403849	BRD4	rs3826863	0.0031	Whole-Blood	[195]

Ethics approval letters

QIMR Berghofer
Medical Research Institute

2 June 2017

Phone: 07 3362 0117
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Chief Investigator:	Surname	Title	Initials
A	FERREIRA	DR	M
B	MARTIN	PROF	N
C	DUFFY	DR	D
D			

Dear Dr Ferreira,

HREC Reference number: **P710**
Project title: **A Twin-Family Study of the Association Between Genes and Asthma**

The annual report for this project submitted on 20 April 2017 was reviewed by the QIMR Berghofer-HREC on 2 June 2017.

The study meets the requirements of the *NHMRC National Statement* and the documents listed below are approved:

- *20 Apr 2017 – P710: 2016 Human Annual Report*

Renewed approval of this project is valid from **2 June 2017** to **1 June 2018** subject to the following conditions being met:

- This QIMR Berghofer-HREC Approval is subject to ethical approval/s from all collaborating HREC/s.
- The Principal Investigator will submit an annual report to the QIMR Berghofer-HREC by no later than 5 weeks prior to approval expiry.
- The Principal Investigator will immediately report anything that might warrant review of ethical approval of the project.
- The Principal Investigator will notify the QIMR Berghofer-HREC of any event that requires a modification to the protocol, including site changes, or other project documents and submit any required amendments in accordance with the ToR provided by the HREC. These instructions can be found at http://www.qimrberghofer.edu.au/about-us/ethics-committees/qimr-berghofer-human-research-ethics-committee/important-hrec-document-downloads/
- The Principal Investigator will submit any necessary reports related to the safety of research participants in accordance with QIMR Berghofer-HREC policy and procedures. These instructions can be found at http://www.qimrberghofer.edu.au/about-us/ethics-committees/qimr-berghofer-human-research-ethics-committee/important-hrec-document-downloads/
- The Coordinating Principal Investigator will notify the QIMR Berghofer-HREC of any plan to extend the duration of the project past the approval period listed above and will submit any associated required documentation.

- The Coordinating Principal Investigator will notify the QIMR Berghofer-HREC of his or her inability to continue as Coordinating Principal Investigator including the name of and contact information for a replacement.

Should you wish to discuss this matter, please contact Marion Marson, HREC Secretariat, on 3362 0117 or HREC.Secretariat@qimrberghofer.edu.au.

The QIMR-Berghofer HREC wishes you every continued success in your research.

Yours sincerely,

Ian Wilkey
QIMR-Berghofer HREC Chair

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) *National Statement on Ethical Conduct in Human Research (2007).* The processes used by this HREC to review multi-centre research proposals have been certified by the National Health and Medical Research Council.

QIMR Berghofer
Medical Research Institute

6 June 2014

Phone: 07 3362 3117
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HREC.Secretariat@qimrberghofer.edu.au

Chief Investigator:	Surname	Title	Initials
A	FERREIRA	DR	M
B	BAIN	DR	L
C			
D			

Dear Manuel,

HREC Reference number: **P2025**
Project title: A clinical trial of tocilizumab in participants with asthma

Thank you for submitting the above research project for single ethical review. This project was first considered by the QIMR Berghofer-HREC at its meeting held on 6 Dec 2013, then again on 6 June 2014 following major amendments to the Protocol.

I am pleased to advise you that the QIMR Berghofer-HREC has granted ethical approval of this research project.

The nominated participating site/s in this project is/are:

- QIMR Berghofer Medical Research Institute
- Princess Alexandra Hospital

The final approved documents are:

Document	Version Date
• Eform submitted 4 June 2014 • 5 May 2014 Approval letter from Metro South HREC • 10 Apr 2014 P2025 Response letter to comments from Metro South HREC review • 10 Apr 2014 P2025 Summary of changes to Protocol and PICF documents • 10 Apr 2014 P2025 Revised protocol (v.1 dated 31 March 2014), including changes in response to Metro South HREC review • 10 Apr 2014 P2025 Revised PICF (v.1 dated 31 March 2014), including changes in response to Metro South HREC review • 3 Dec 2013 P2025 Response to Comments • 3 Dec 2013 P2025 IB Tocilizumab, v1 dated 3December 2013	

Approval of this project from QIMR Berghofer-HREC is valid from **6 June 2014** to **5 June 2015** subject to the following conditions being met:

- This QIMR Berghofer-HREC Approval is subject to ethical approval/s from all collaborating HREC/s.

- The Principal Investigator will submit an annual report to the QIMR Berghofer-HREC by no later than 5 weeks prior to approval expiry.
- The Principal Investigator will immediately report anything that might warrant review of ethical approval of the project.
- The Principal Investigator will notify the QIMR Berghofer-HREC of any event that requires a modification to the protocol, including site changes, or other project documents and submit any required amendments in accordance with the instructions provided by the HREC. These instructions can be found at http://intranet.qimr.edu.au/intranet/scientific/ethics/humans/index.html
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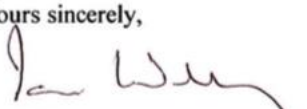
- The Coordinating Principal Investigator will notify the QIMR Berghofer-HREC of any plan to extend the duration of the project past the approval period listed above and will submit any associated required documentation.
- The Coordinating Principal Investigator will notify the QIMR Berghofer-HREC of his or her inability to continue as Coordinating Principal Investigator including the name of and contact information for a replacement.

Should you have any queries about the QIMR Berghofer-HREC's consideration of your project please contact Marion Marson, HREC Secretariat, on 3362 3117 or HREC.Secretariat @qimrberhofer.edu.au. The QIMR Berghofer-HREC's Terms of Reference, Standard Operating Procedures and membership are available from http://intranet.qimr.edu.au/intranet/scientific/ethics/humans/index.html.

The QIMR Berghofer-HREC wishes you every success in your research.

Yours sincerely,



Ian Wilkey
QIMR-Berghofer HREC Chair

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) *National Statement on Ethical Conduct in Human Research (2007)*. The processes used by this HREC to review multi-centre research proposals have been certified by the National Health and Medical Research Council.

QIMR Berghofer
Medical Research Institute

24 April 2015

Phone: 07 3362 0117
Fax: 07 3362 0109
E-mail:
HREC.Secretariat@qimrberghofer.edu.au

Chief Investigator:	Surname	Title	Initials
A	FERREIRA	DR	M
B	BAIN	MS	L

Dear Dr Ferreira,

HREC Reference number: **P2103**
Project title: **McMaster data and sample collection - A P2025 substudy**

Thank you for submitting the above research project for single ethical review. This project was considered by the QIMR Berghofer-HREC at its meeting held on 24 April 2015.

I am pleased to advise you that at this meeting the QIMR Berghofer-HREC granted ethical approval of this research project.

The approved documents are:

Document	Version submitted
E-Form P2103	23 March 2015
• *23 Mar 2015 - P2103 cover letter 23.03.2015* • *20 Mar 2015 - P2103 Study Protocol* • *20 Mar 2015 - HIREB approval letter* • *20 Mar 2015 - Health Canada - NOL* • *20 Mar 2015 - Genetic ICF Tocilizumab 15OCT2014* • *20 Mar 2015 - ICF Tocilizumab 15JAN2015* • *20 Mar 2015 - TCZ001 Protocol 01SEP2014*	

Approval of this project from QIMR Berghofer-HREC is valid from **24 April 2015** to **23 April 2016** subject to the following conditions being met:

- This QIMR Berghofer-HREC Approval is subject to ethical approval/s from all collaborating HREC/s.
- The Principal Investigator will submit an annual report to the QIMR Berghofer-HREC by no later than 5 weeks prior to approval expiry.
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The QIMR Berghofer-HREC wishes you every success in your research.

Yours sincerely,

K. R. Trenholme.

A/Prof Katharine Trenholme
QIMR-Berghofer HREC Acting Chair

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) *National Statement on Ethical Conduct in Human Research (2007)*. The processes used by this HREC to review multi-centre research proposals have been certified by the National Health and Medical Research Council.

Hamilton Health Sciences

McMaster University
Inspiring Innovation and Discovery

St. Joseph's Healthcare Hamilton

Hamilton Integrated Research Ethics Board (HIREB)
293 Wellington St. N., Suite 102, Hamilton, ON L8L 8E7
Telephone: 905-521-2100, Ext. 42013
Fax: 905-577-8378

March 2, 2015

PROJECT NUMBER: **14-790**

PROJECT TITLE: A double-blind, placebo controlled, randomised study to evaluate the safety and efficacy of a single intravenous dose of Tocilizumab for the treatment for allergen-induced asthma

PRINCIPAL INVESTIGATOR: **Dr. Gail Gauvreau**

This will acknowledge receipt of your letter dated January 15, 2015 which enclosed revised copies of the Information/Consent Forms, Protocol, Outstanding Signatures and the Application Form along with response to the additional queries of the Board for the above-named study. These issues were raised by the Hamilton Integrated Research Ethics Board at their meeting held on November 18, 2014. Based on this additional information, we wish to advise your study has been given ***final*** approval from the full HIREB.

The following documents have been approved on both ethical and scientific grounds:

- The submission
- Clinical Study Protocol TCZ001 version 1 dated September 1, 2014
- Information/Consent Form version 2 dated January 15, 2015
- Genetic Information/Consent Form version 1 dated October 15, 2014
- Investigator's Brochure for Tocilizumab for Treatment of asthma version 1 dated October 15, 2014

The following documents have been acknowledged:

- Health Canada No Objection Letter dated December 24, 2014; Control # 179785
- Clinical Trial Registration # ACTRN12614000123640
- Metro South Health Human Research Ethics Committee Amendment Approval dated July 29, 2014
- QIMR Berghofer Medical Research Institute Amendment Approval dated July 17, 2014
- QIMR Berghofer Medical Research Institute Amendment Approval dated July 25, 2014

Please note attached you will find the Information/Consent Forms with the HIREB approval affixed; all consent forms used in this study must be copies of the attached materials.

The Hamilton Integrated Research Ethics Board operates in compliance with and is constituted in accordance with the requirements of: The Tri-Council Policy Statement on Ethical Conduct of Research Involving Humans; The International Conference on Harmonization of Good Clinical Practices; Part C Division 5 of the Food and Drug Regulations of Health Canada, and the provisions of the Ontario Personal Health Information Protection Act 2004 and its applicable Regulations; for studies conducted at St. Joseph's Hospital, HIREB complies with the health ethics guide of the Catholic Alliance of Canada

REB #: 14-790 Gauvreau

We are pleased to issue final approval for the above-named study for a period of 12 months from the date of the HIREB meeting on November 18, 2014. Continuation beyond that date will require further review and renewal of HIREB approval. Any changes or revisions to the original submission must be submitted on an HIREB amendment form for review and approval by the Hamilton Integrated Research Ethics Board.

PLEASE QUOTE THE ABOVE-REFERENCE PROJECT NUMBER ON ALL FUTURE CORRESPONDENCE

Sincerely,

S. Salama

Suzette Salama, PhD.
Chair, Hamilton Integrated Research Ethics Board

QIMR Berghofer
Medical Research Institute

3 November 2016

Phone: 07 3362 0117
Fax: 07 3362 0109
E-mail:
HREC.Secretariat@qimrberghofer.edu.au

Chief Investigator:	Surname	Title	Initials
A B	FERREIRA	DR	M

Dear Dr Ferreira,

HREC Reference number: P2242
Project title: Frequency of asthma prescriptions among patients on regular tocilizumab treatment - pilot study

Thank you for submitting the above research project for single ethical review. This project was considered by the QIMR Berghofer-HREC at its meeting held on 26 August 2016 and subsequently granted approval by the HREC Chair out of session on 3 November 2016.

Please provide a copy of the final AIHW study approval once obtained, thank you.

The documents listed below are approved:

- *1 Nov 2016 - Provisional approval by AIHW committee (email from Kate Phillips)*
- *16 Aug 2016 – P2242 E-form application*
- *20 July 2016 – P2242 Protocol July 2016*

Approval of this project from QIMR Berghofer-HREC is valid from **3 November 2016** to **2 November 2017** subject to the following conditions being met:

- This QIMR Berghofer-HREC Approval is subject to ethical approval/s from all collaborating HREC/s.
- The Principal Investigator will submit an annual report to the QIMR Berghofer-HREC by no later than 5 weeks prior to approval expiry.
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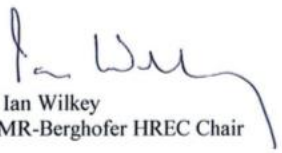
The QIMR Berghofer-HREC wishes you every success in your research.

Yours sincerely,



Dr Ian Wilkey
QIMR-Berghofer HREC Chair

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