Genetic determinants of vitamin D status and susceptibility to acute respiratory infection

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Submitted in partial fulfilment of the requirements of the degree of Doctor of Philosophy

Statement of Originality

I, David Anthony Jolliffe, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

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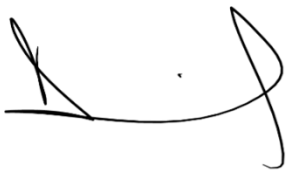
Signature: 

Date: 29th February 2016

Details of collaborations:

I worked with the three clinical trial study teams from the outset and aided in the recruitment and follow-up of participants, which included: collection of anthropometric, lifestyle, and disease phenotype data by questionnaire; collection of disease phenotype data by clinical procedures e.g. lung function and quadriceps strength tests; collection, separation, and storage of blood samples; and collection of induced sputum samples.

I selected my panel of SNP, designed custom TaqMan assays, and conducted the genotyping of clinical trial participants under the supervision of Dr Mimoza Hoti (Queen Mary University's Genome Centre).

In collaboration with Dr Jennifer Roe (Division of Infection & Immunity, University College London) I carried out RNA extraction and RT-qPCR on a subset of participants from the ViDiCO trial.

I assisted Dr. Claire Greiller (Blizard Institute, QMUL, UK) in the *ex vivo* stimulation of whole blood samples, and aspiration of supernatants for freezer storage and subsequent 30-plex ELISAs. I also conducted whole blood and PBMC assays on clinical trial samples collected in Dr. Greiller's absence.

Biochemical analyses for the 3 clinical trials to determine serum concentrations of 25-hydroxyvitamin D, albumins, calcium, and parathyroid hormone were carried out in the Department of Clinical Biochemistry at Homerton Hospital, by Ms Marion Rowe and Dr Peter Timms.

Primary analysis of clinical trial results (time to ARI/exacerbation) was performed by Professor Adrian Martineau (Blizard Institute, QMUL, UK)

All cross-sectional, main effects, interaction and meta-analyses presented in this thesis were performed by myself.

Abstract.

Introduction: Acute respiratory infections (ARI) are a major global cause of morbidity and mortality. Vitamin D deficiency has been reported to associate with susceptibility to ARI and with greater severity and poorer control of asthma and chronic obstructive pulmonary disease (COPD). Clinical trials of vitamin D for the prevention of ARI have yielded heterogeneous results, with some showing protection and others not. This may reflect variation in the frequency of genetic variants influencing response to vitamin D supplementation in different populations. The impact that genetic variation in the vitamin D pathway has on vitamin D status, disease phenotype and response to vitamin D supplementation in prevention of ARI has not been comprehensively investigated.

Methods: I conducted:

1. A systematic review and meta-analysis of clinical studies which have investigated vitamin D as a potential therapy for ARI;

2. Three cross-sectional studies (in n=297 adult asthma patients, n=278 COPD patients, and n=272 older adults) to investigate potential environmental determinants (lifestyle and anthropometric) and genetic determinants (35 single nucleotide polymorphisms [SNP] in 11 vitamin D related genes) of serum 25-hydroxyvitamin D concentration (25[OH]D) and clinical phenotype;

3. Three prospective studies investigating the influence of genetic variation in the vitamin D pathway on a) susceptibility to ARI (main effects analysis) and b) efficacy of vitamin D supplementation for the prevention of ARI (interaction analysis).

Results: My systematic review identified consistent reports of an inverse association between vitamin D status and risk of ARI in observational studies, and heterogeneous reports from clinical trials. My cross-sectional studies identified a range of classical environmental factors which predict vitamin D status in the three study populations, but did not identify any genetic variants in the vitamin D pathway that associate with vitamin D status. I identified an association between vitamin D deficiency and decreased lung function in COPD patients, but no associations between vitamin D deficiency and

asthma phenotype. Finally, my analysis identified a haplotype of 5 single nucleotide polymorphisms in the vitamin D receptor (VDR) gene which significantly modify the effect of vitamin D supplementation on risk of upper respiratory infection in COPD patients.

Conclusions: I identified environmental determinants that predict 25(OH)D concentrations in all three study populations, but only found an association between vitamin D deficiency and disease severity in COPD patients. Furthermore, I identified a haplotype in *VDR* which modifies the effect of vitamin D supplementation in COPD patients to result in a significantly reduced risk of ARI.

Table of Contents.

List of Tables

List of Figures

List of abbreviations.

$1,25(OH)_2D$	1,25-dihydroxyvitamin D; calcitriol
25(OH)D	25-hydroxyvitamin D; calcidiol
7-DHC	7-dehydrocholesterol
aHR	Adjusted hazard ratio
AMP	Antimicrobial peptide
aRHR	Adjusted ratio of hazard ratios
ARI	Acute respiratory infection
BMI	Body mass index
BTS	British thoracic society
CD	Cluster of differentiation
cDNA	Complementary DNA
CGAS	Candidate gene association study
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
C_t	Cycle threshold
CUBN	Cubilin
CXCL	Chemokine (C-X-C motif) ligand
CYP2R1	Cytochrome p450, family 2, subfamily R, polypeptide 1
CYP24A1	Cytochrome p450, family 24, subfamily A, polypeptide 1; 24 hydroxylase
CYP27A1	Cytochrome p450, family 27, subfamily A, polypeptide 1
CYP27B1	Cytochrome p450, family 27, subfamily B, polypeptide 1; 1 alpha hydroxylase
CYP3A4	Cytochrome p450, family 3, subfamily A, polypeptide 4
DBP	Vitamin D binding protein; Group-specific Component (Gc)
DC	Dendritic cell
DHCR7	7-dehydrocholesterol reductase
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
Eotaxin *	CCL11
FeNO	Fractional exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 second
FGF-basic	Basic fibroblast growth factor
FVC	Forced vital capacity
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
G-CSF	Granulocyte colony-stimulating factor
GINA	Global Initiative for Asthma guidelines
GM-CSF	Granulocyte macrophage colony-stimulating factor
GMR	Geometric mean ratio
GOLD	Global initiative for chronic obstructive lung disease
GWAS	Genome-wide association study
HGF	Hepatocyte growth factor
HR	Hazard ratio
ICS	Inhaled corticosteroid
iDC	Immature dendritic cell
IFN	Interferon
Ig	Immunoglobulin

IL	Interleukin
IMP	Investigational medicinal produce
IP-10	Interferon gamma-induced protein 10; CXCL10
IQR	Interquartile range
IU	International units
LABA	Long-acting beta-adrenoceptor agonists
LPS	Lipopolysaccharide
LRI	Lower respiratory tract infection
LRP2	Low density lipoprotein receptor-related protein-2 (Megalin)
MCP-1	Monocyte chemotactic protein-1; CCL2
mDC	Myeloid dendritic cell
MHC	Major histocompatibility complex
MIG	Monokine induced by gamma interferon; CXCL9
MIP-1α	Macrophage inflammatory protein-1α; CCL3
MIP-1β	Macrophage inflammatory protein-1β; CCL4
mRNA	Messenger RNA
M_ϕ	Macrophage
NHS	National health service
OCS	Oral corticosteroids
OR	Odds ratio
Pam2	Pam2CSK4
Pam3	Pam3CSK4
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PEFR	Peak expiratory flow rate
polyI:C	Polyinosinic polycytidylic acid
PPV	polysaccharide pneumococcal vaccine
ppb	Parts per billion
PTH	Parathyroid hormone
QoL	Quality of life
QS	Quadriceps strength
R848	Resiquimod
RANTES	Regulated on activation, normal T cell expressed and secreted; CCL5
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RR	Risk ratio
RSV	Respiratory syncytial virus
RT-qPCR	Reverse transcriptase-quantitative polymerase chain reaction
RV	Rhinovirus
RXRA	Retinoid X Receptor
SD	Standard deviation
SEP	Socio-economic position
SGRQ	St George's respiratory questionnaire
SNP	Single nucleotide polymorphism
TGF	Transforming growth factor
Th	T helper cell
TLR	Toll-like receptor

TNF	Tumour necrosis factor
Treg	Regulatory T-cell
UD	Undefined
URI	Upper respiratory tract infection
UVR	Ultraviolet radiation
VDR	Vitamin D receptor
VDRE	Vitamin D response element
VEGF	Vascular endothelial growth factor
ViDiAs	Trial of vitamin D supplementation in asthma
ViDiCO	Trial of vitamin D supplementation in COPD
ViDiFlu	Trial of vitamin D supplementation for the prevention of ARI
WBA	Whole blood assay
WHO	World health organisation
$\chi 2$	Chi-squared

1. Introduction.

This introductory chapter offers an overview of the epidemiology of acute respiratory infection (ARI); a summary of the vitamin D metabolic pathway and mechanisms by which vitamin D may prevent ARI; and a review of genetic variation in the vitamin D pathway, and how this may influence susceptibility to ARI, either as a main effect or as a modifier of the effects of vitamin D supplementation.

1.1. Acute Respiratory Infection.

1.1.1. Definition, Classification and Aetiology of ARI.

Acute respiratory infections (ARI) are defined as infections of one or more sites along the respiratory tract, comprising the paranasal sinuses, nasal cavity, pharynx, larynx, epiglottis, trachea, bronchioles and lungs, with symptoms lasting for 30 days or less. ARI are classified as either upper respiratory infection (URI), or lower respiratory infection (LRI) according to their localisation above or below the vocal cords, respectively.

Upper respiratory infection.

URI are the most common infections in humans. The most typical clinical manifestation of URI is the common cold, which is often referred to as a "symptom-complex" as multiple sites along the upper respiratory tract become infected, while other common syndromes (laryngitis, pharyngitis, tonsillitis and otitis media) occur at a single site and like the common cold are considered mild, self-limiting infections that are predominantly viral in origin. Rhinoviruses are the commonest causative agent of viral URI, accountable for roughly 30% of infections; respiratory syncytial virus (RSV), human parainfluenza & influenza viruses, human meta-pneumovirus, and adenoviruses together are believed to cause up to 30%; coronaviruses are responsible for 10%, whilst the remaining ~30% of URI are thought to be of unknown viral aetiology (1). URI are less frequently caused by bacteria: severe cases of laryngotracheitis and epiglottitis are typically caused by *Haemophilus influenzae* type B. Furthermore, the previously mentioned viral syndromes of URI (laryngitis, pharyngitis, tonsillitis and otitis media) can also to a lesser extent be caused by a range of bacteria: Group A *streptococci, Mycoplasma pneumonia, Neisseria gonorrhoeae, Staphylococcus aureus* and *Streptococcus pneumonia* are the most frequently identified agents (2).

Lower respiratory infection.

Syndromes of acute lower respiratory infection (influenza-like illness [which also affects the upper respiratory tract], bronchitis, bronchiolitis and pneumonia) are less frequent than URI, though they have more severe health implications and as such are one of the leading causes of mortality worldwide. LRI syndromes may also have a viral, bacterial or fungal aetiology, and in some instances e.g. community acquired pneumonia, there occurs a dual viral, dual bacterial, or mixed viral-bacterial aetiology (3). Influenza-like illness is caused by the influenza virus (types A, B and C) which belong the Orthomyxoviridae family. Influenza A and B are largely responsible for seasonal influenza epidemics, whilst species of type A influenza virus have been responsible for past human pandemics (4). Bronchitis and bronchiolitis have both bacterial and viral aetiologies. Bronchitis often occurs as a secondary complication of a viral URI; the most commonly detected viruses are RSV, human parainfluenza virus and coronavirus (5), though the bacteria *Mycoplasma pneumonia*, *Chlamydia pneumoniae* and *Bordetella pertussis* have also been identified (6). Bronchiolitis is generally considered a paediatric syndrome and as with bronchitis, the most commonly isolated pathogen (in up to 90% of cases) is RSV. Rhinovirus and human meta-pneumovirus have also been implicated, and in rare cases, *Mycoplasma pneumonia* (7). Pneumonia is responsible for a large proportion of LRI-related deaths. It can be caused by a wide variety of respiratory pathogens, though unlike the majority of the previously mentioned syndromes, it typically has a bacterial aetiology*: Streptococcus pneumoniae* is the most common causative agent in children, followed by *Haemophilus influenzae* type B (8). Other isolated bacteria include *Staphylococcus aureus*, *Klebsiella pneumonia, Escherichia coli, Mycoplasma Pneumoniae, Pseudomonas aeruginosa* and *Moraxella catarrhalis*. In terms of viral pneumonia, RSV is the most common causative agent, though influenza, rhinovirus and parainfluenza viruses can also be responsible (9). In addition to these pathogens, fungal species can cause endemic pneumonia outbreaks in environmental niches with conditions that favour fungal growth (10), as well as being dangerous opportunist pathogens for the immunocompromised. The species: *Aspergillus*, *Candida*, *Cryptococcus, Histoplasma capsulatum* and *Coccidioides* are common agents, and in HIV-

infected infants, *Pneumocystis jiroveci* is responsible for one quarter of pneumonia-related deaths (8, 11).

1.1.2. Pathogenesis of ARI

The respiratory tract is the route of access most commonly exploited by invading pathogens owed to it being constantly exposed to our external environment. For example, an individual living in the Midwest of the United States has been estimated to inhale 860,000 bacteria per day (12), which does not include respiratory viruses that are responsible for the majority of ARI. Most pathogens which invade the respiratory tract are adequately combated by host innate and adaptive immune responses. However, in some cases an invading pathogen is able to effectively circumvent or impede an immune response. The specific strategies employed by respiratory pathogens are numerous. Some examples include: hiding from immune surveillance, generating antigenic hyper-variability, inhibiting the complement pathway, directly inhibiting the release of cytokines, chemokines, and interferons from effector immune cells, modulation of apoptosis, and the interference of toll-like receptor proteins (13). These tactics often allow the pathogen to colonise the respiratory tract and directly inflict damage to pulmonary architecture. A degree of indirect damage is also caused by the immune response to the pathogen (immunopathology). Which source of damage most significantly contributes to the precipitation of clinical sequelae is not well understood because their effects are difficult to differentiate, and they vary by pathogen (14). To use RSV as an example: it causes direct viral damage by inhibition of ciliated epithelium movement to cause airway blockade (15). Indirectly, RSV affects immune-mediated pathology by causing a hyper-inflammatory state that arises due to a large influx of leukocytes. Neutrophils are often the most commonly found leukocyte at the site of infection and cause overstimulation of inflammatory factors and cytotoxic enzymes which result in airway tissue damage (16). Both of these forms of damage combine to cause increased mucus production, cell

apoptosis, tissue necrosis, and sloughing of epithelial cells (17), often resulting in symptoms of increased mucus secretion, difficulty in breathing, nasal congestion, fever, and coughing.

The different forms of direct pathogenic damage and immunopathology are vast due to the wide range of pathogens that cause ARI, but in general, the degree of infection reflects the level of structural damage and functional compromise caused to pulmonary architecture, which is mostly dependent on the amount of pathogen dissemination along the respiratory tract.

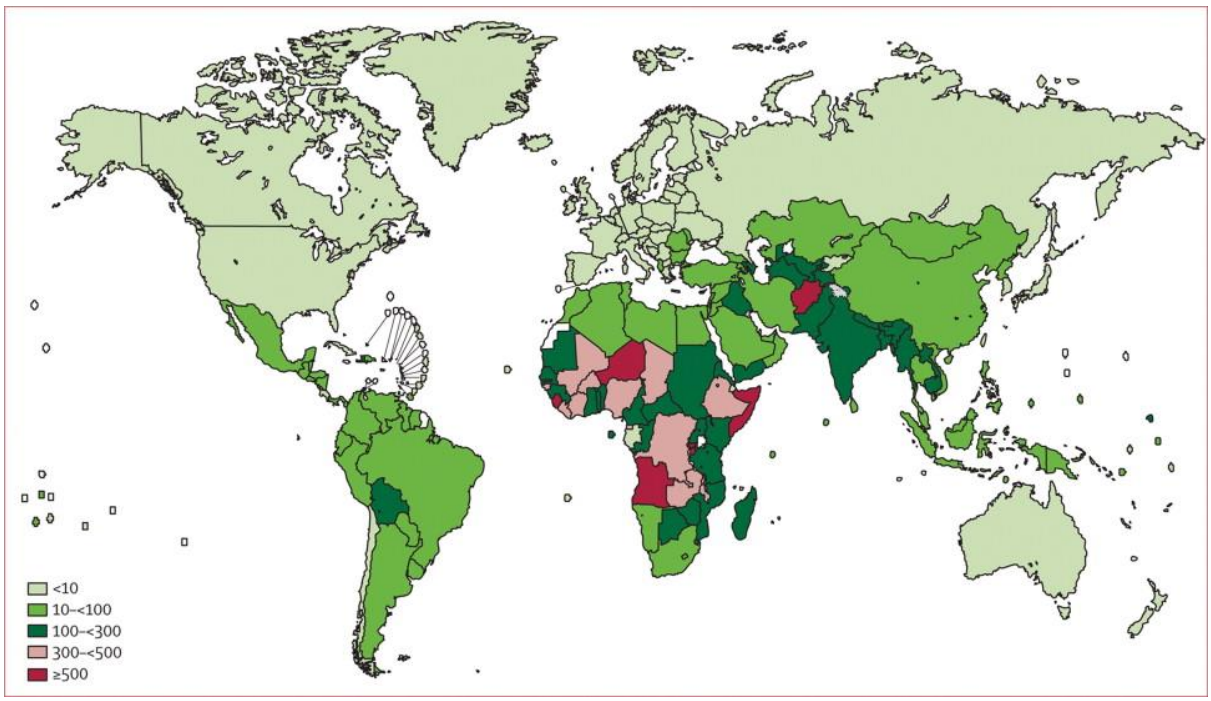
1.1.3. The Burden of ARI: Economics & Mortality.

As previously mentioned, LRI carry a far greater risk of death than do URI. The recently published 'Global Burden of Disease Study 2013' attributed 3.9 thousand deaths globally to URI in 2013, and 2.7 million deaths to LRI (18). URI generally impose a large burden on the economy through healthcare related costs and loss of productivity due to work and school absenteeism. In many developed countries there has been a burgeoning health economics issue surrounding the over-prescription of antibiotics in primary care settings, for the treatment of URI which are predominantly self-limiting viral infections (19). Between 1980 and 1991, there was an estimated 46% increase in the number of antibiotic prescriptions issued in England (20); a 65% increase in France; and a 78% increase in West Germany (21). Between 1994 and 2000, in the UK, antibiotic prescription incidence reportedly decreased significantly (22), though a recently published survey found the median general practice (of 568 providing electronic medical records) prescribed antibiotics for 54% of URI consultations in 2010 and 2011 (23). It has been estimated that £15 million per year is spent in the UK on antibiotics prescribed in primary care settings for patients presenting only with acute coughs (24), furthermore, work absence due to coughs and colds during 2011 were estimated at a total of 27.5 million days (25). A similar story has been observed in the US: in 1998, 55% (22.6 million) of antibiotic prescriptions were

made for URI, at a cost of roughly $726 million (26). In 1996 upper respiratory infections were estimated to cause 20 million days of work absence, and 22 million days of school absence (27). The overprescription of antibiotics to treat viral URI, which occurs in many parts of the world, also contributes to the economic burden imposed by ARI. This is not only due to the immediate cost of prescribed antibiotics, but also due to the investment in solutions to combat the resulting problem which has arisen from overprescription: the widespread use of antibiotics has generated a strong selective pressure for strains of bacteria that can adapt, and as such antibiotics which were once very effective in the management of ARI have had to be replaced by, or combined with other forms of antibiotic. Penicillin in the treatment of *Streptococcus pneumoniae* infection is a classic example. *S. pneumoniae* is one of the most frequent causes of pneumonia which until the mid-1960s was universally sensitive to penicillin treatment. The first case of penicillin-resistant *S.pneumoniae* was identified in 1967, in Australia, and 10 years later within an infectious disease hospital in South Africa where patients were routinely prescribed antibiotics as prophylaxis for viral infection, strains of *S.pneumoniae* with additional resistance to erythromycin, tetracycline, streptomycin and clindamycin were reported (28). By 1997 an estimated 14% of *S.pneumoniae* isolates had achieved penicillin resistance, globally (29), which had increased to 22% of isolates in the US by 2004 (30), and as much as 50% in other parts of the world (31). Due to the development of drug-resistant pathogens it has been estimated that from 1991 to 2012, in the UK, there has been a 12% increase in failure rates for first line antibiotic treatment of upper and lower respiratory infections, and soft tissue infections (32).

The burden of ARI mortality is predominantly due to pneumonia, and is particularly high in people on either end of the age scale – the very young and the very old. Between 2000 and 2003, pneumonia was estimated to be the single largest cause of child mortality globally in those aged <5 years; responsible for 19% of deaths (2.01 million) each year (33), and as figure.1 shows, mortality rates were highest in sub-Saharan Africa and South Asia.

Figure 1.1 shows the WHO's estimations for global pneumococcal mortality in 2000, for children aged 1-59 months per 100,000 children aged <5 years old, per year (34).



Mortality rates in children aged <5 years have decreased significantly in the last decade, which may be due to increased vaccination efforts or more effective treatment strategies, and perhaps other factors such as improvements in socioeconomic status and nutrition, or the decreasing prevalence of smoking have contributed to the effect. Despite this decline, according to the WHO, in 2013 pneumonia was still the global leading cause of infectious mortality in this age-group; responsible for an estimated 0.94 million deaths (8). In older adults, impaired response to influenza vaccines have been reported (35) and controversy surrounds the efficacy of the 23-valent polysaccharide pneumococcal vaccine (PPV) introduced in Europe in 1998 for vaccination against pneumococcal pneumonia in those aged >65 years: while some clinical trials have reported a reduced incidence of infection and mortality and sustained immunogenicity (36, 37), survey data still reports that 90% of deaths in Europe caused by pneumonia remain in the over 65 age-group (38).

The burden of ARI is also particularly high in those with chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD). URI have been reported to be the major precipitant of symptom exacerbations in children and adults with asthma (39); a chronic respiratory disease which causes roughly 250,000 deaths globally each year (40) and significant morbidity in the estimated 235 million worldwide sufferers (41). In the UK, there are approximately 5.1 million sufferers and an estimated 12.7 million lost work days per year; costing the economy £2.3 billion in lost productivity, welfare benefits, and NHS costs (42). COPD represents an even larger global health problem which is expected to worsen in the coming decade. The disease was responsible for over 3 million deaths in 2012 (43), and an estimated $32.1 billion in medical care costs during 2010, for the US alone (44). It is estimated that 50% of COPD exacerbations are precipitated by URI (45), therefore more effective interventions to prevent ARI could have significant, positive health and economic implications for patients with both of these respiratory diseases.

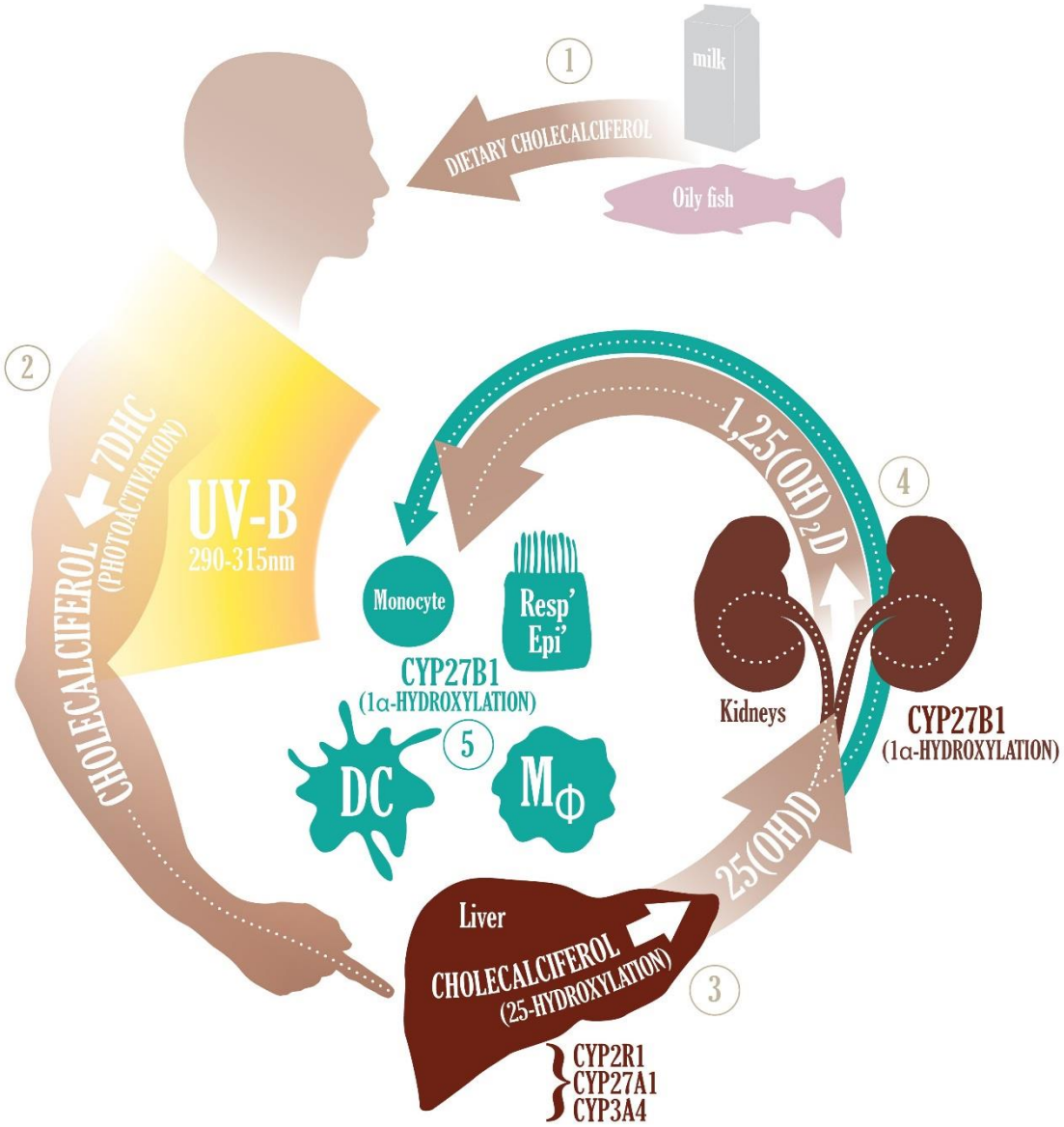
1.2. Vitamin D.

In the early 20th century it was discovered that increased sun exposure and the administration of cod-liver oil could be used to treat rickets (46). Although it was not known at the time that improvement of vitamin D status was the feature these two treatments had in common, it has since become well known that vitamin D is essential for the regulation of calcium and phosphate homeostasis to maintain skeletal bone health. More recently vitamin D's potential 'non-classical' roles have begun to emerge, one of them being its immunomodulatory activity.

1.2.1. Biochemistry and metabolism.

In 1914 a team of biochemists in the US lead by Elmer V. McCollum isolated a substance from butterfat they termed 'fat-soluble factor A' (47). Through further experimentation with heat oxidised cod-liver oil, in 1922 McCollum reported the discovery of a distinct compound in fat-soluble factor A, which was later named 'vitamin D' (48). The name belies its chemical nature however: due to a broken carbon-carbon bond in its B ring and the requirement of a change in the compound's structure before it can become metabolically active, it is more accurate to regard vitamin D as a secosteroid pre-prohormone. This has not prompted a change in nomenclature however; the term vitamin D is still generally used to refer to the two known forms of the compound - vitamin D_2, (ergocalciferol) which is derived from ergosterol, a cell wall component found in fungi, or vitamin D_3 (cholecalciferol), which is derived from 7-dehydrocholesterol (7-DHC), a zoosterol produced by the skin (49).

Figure 1.2. A diagram depicting the intake of dietary vitamin D (1) from two common oral sources, fortified milk and oily fish; the cutaneous synthesis of vitamin D (2) via photoactivation of 7-dehydrocholesterol by ultra-violet radiation which occurs in the skin; the 25-hydroxylation of cholecalciferol (3) by cytochrome P450 enzymes in the liver to produce the intermediate metabolite of vitamin D, 25(OH)D; the 1α-hydroxylation of 25(OH)D in the kidneys (4) by CYP27B1 enzyme to produce the active metabolite of vitamin D, 1,25(OH)$_2$D$_3$; the extra-renal 1α-hydroxylation of 25(OH)D (5) which occurs in a variety of cells, but of particular interest to immune responses to respiratory pathogens, this occurs in monocytes, respiratory epithelial cells, dendritic cells (DC) and macrophages (M$_\varphi$) via intra-cellular expression of the CYP27B1 enzyme.



Humans synthesize cholecalciferol in the skin following exposure to ultra-violet B (UV-B) radiation, though we also receive some from our diet (primarily from oily fish [pathway.1 in Figure 1.2]). The cutaneous production of cholecalciferol is achieved by photo-activation of 7-DHC when UV-B rays with a wavelength of 290 to 315 nm penetrate the epidermal and dermal layers: a photon of radiation is absorbed by 7-DHC which cleaves the B ring's C_9-C_{10} bond to create a seco-sterol, called pre-vitamin D_3 (50). Pre-vitamin D_3 then undergoes spontaneous thermal isomerisation to become vitamin D_3 (cholecalciferol) (pathway.2 in Figure 1.2) (51). Vitamin D_3 is then hydroxylated twice by cytochrome P450 enzymes to produce an active metabolite. The first reaction primarily occurs in the liver (pathway.3, Figure 1.2), predominantly by CYP2R1, though CYP27A1 and CP3A4 have also been reported to show 25-hydroxylation activity (52, 53). This reaction produces 25-hydroxyvitamin D_3 ($25[OH]D_3$, also referred to as 'calcidiol') - the intermediate, major circulating vitamin D metabolite and widely accepted measure of vitamin D status due to its longer half-life than the metabolically active form of vitamin D (ca.15 days vs. 15 hours) (54). The second reaction occurs in the kidneys and a variety of extra-renal tissues around the body, including certain innate immune cells (pathways 4 & 5, Figure 1.2). The enzyme CYP27B1 1α-hydroxylates $25(OH)D_3$ to produce 1α,25-dihydroxyvitamin D_3 ($1,25[OH]_2D_3$, also referred to as 'calcitriol') (55). This is the active metabolite of vitamin D which ligates vitamin D receptor (VDR) with high affinity to mediate the secosteroid hormone's actions. To avoid excess concentration of the active metabolite, $1,25(OH)_2D_3$ down-regulates its own production by VDR-mediated negative regulation of *CYP27B1* gene (56, 57) and catabolism of vitamin D also occurs by the enzyme CYP24A1, which can 24R-hydroxylate $25(OH)D_3$ and $1,25(OH)_2D_3$ to produce the relatively inactive metabolites $24,25(OH)_2D_3$ and $1,24,25(OH)_3D_3$, respectively; either indirectly, or directly decreasing the amount of active substrate available for reaction (58). Excessive supplementation of vitamin D can result in toxicity (hypervitaminosis D), the major symptoms of which arise from elevated calcium levels: nausea, constipation, dehydration, excessive thirst and vomiting, and in some cases, nocturia, muscle weakness, itching and renal stones (59). If left untreated, hypervitaminosis D can result in renal failure, however, due to vitamin D's wide therapeutic index,

toxicity in general is relatively rare. There are currently no population-level statistics on the incidence of vitamin D toxicity; existing data comes from case studies (60) (61) (62) and reports of adverse events from clinical trials of vitamin D supplementation: to illustrate, a recently completed individual patient data meta-analysis conducted by my group reports a total of 3 cases of hypercalcaemia, and 2 cases of renal stones in 5,703 members of the intervention arms, of 24 clinical trials. Hypervitaminosis D has been suggested to arise when serum 25(OH)D_3 concentrations rise above 375 nmol/L (63). However to achieve this would require an estimated sustained supplementation of 40,000 IU per day (64), which is far in excess of safe upper dosing limits for clinical trials, currently set at 2,500 IU per day for 1-3 year olds; 3,000 IU per day for 4-8 year olds; and 4,000 IU per day for those aged ≥9 years (65).

1.2.2. Vitamin D deficiency & susceptibility to Acute Respiratory Infections.

The association of sunlight with well-being long predates modern medicine - throughout human history ancient cultures have worshiped solar deities, whom they credited with powers of healing, such as the Mesopotamian God, Shamash; the Celtic God, Belenus; and the Greek God, Apollo. Whilst unempirical, it is interesting to speculate that humans made a connection between sunlight and improved health more than two millennia before the work of Niels Ryberg Finsen, a Faroese born physician who in the late 1880's devoted his research to deciphering the clinical benefits of the sun after observing an improvement in the symptoms of his own condition (Pick's disease) with increased sun exposure. Finsen was awarded the Nobel Prize in Physiology or Medicine in 1903 for demonstrating that ultra-violet light therapy is effective in the treatment of cutaneous tuberculosis (66). Roughly 80 years later the British physician-turned-epidemiologist, Robert Edgar Hope-Simpson proposed a theory to explain the seasonal cycle of epidemic influenza we see in temperate latitudes. His 'seasonal stimulus' theory attributed the fluctuating incidence of influenza across seasons to be "*a rather direct effect of some component of solar radiation acting positively or negatively upon the virus, the human host, or their interaction"(67).* Hope-Simpson's work paved the way for the investigation

of this component of solar radiation which may modulate immune responses to respiratory pathogens.

1.2.3. Immunomodulatory actions of vitamin D.

Over the past 10 years a wide range of primarily *in vitro* data has been published reporting the actions of $1,25(OH)_2D_3$ on both the innate and adaptive arms of the immune system. These have been summarised in a simplified diagram (figure.3) below.

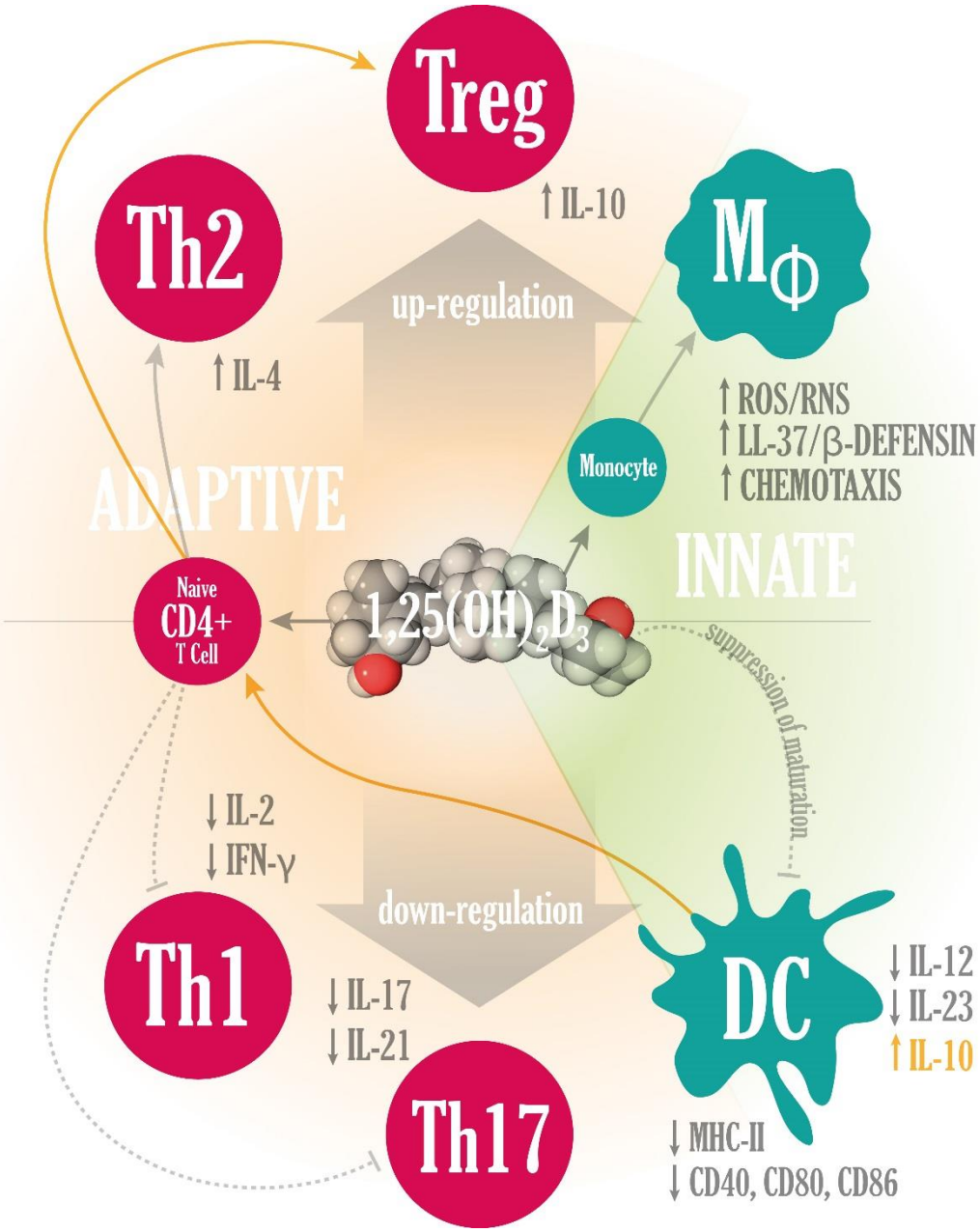
Effect of vitamin D on innate immune responses.

In the innate immune system $1,25(OH)_2D_3$ has been found to: induce expression of antimicrobial peptides (AMPs, e.g. cathelicidin LL-37 and beta-defensins) in monocytes, macrophages and respiratory epithelial cells, providing them with increased bactericidal, virucidal and fungicidal properties (68-71); enhance monocyte and macrophage antimicrobial effector function through induction of reactive oxygen and nitrogen intermediate production (72-74); promote chemotaxis in neutrophils, macrophages, monocytes and T Cells via induction of cathelicidin LL-37 (75); and strengthen barriers to infection by inducing synthesis of structural proteins which form tight-, adherens- and gap-junctions between epithelial cells (76-78) (depicted by the green area in Figure 1.3).

$1,25(OH)_2D_3$ also directly affects dendritic cells (DC) by inhibiting synthesis of their surface receptor (major histocompatibility complex II [MHC-II]) and co-stimulatory proteins (CD40, CD80, CD86) resulting in suppression of maturation and proliferation (79, 80). Immature dendritic cells (iDC) then present antigen to naïve CD4+ T cells to promote tolerance via differentiation to T-regulatory cells

(Tregs) (81) resulting in greater production of anti-inflammatory interleukin-10 (IL-10), thus bridging the effects of innate and adaptive immune responses.

Figure 1.3. An overview of the main actions of $1,25(OH)_2D_3$ on myeloid (green) and lymphoid (red) immune cells in innate and adaptive immune systems. In the innate immune system $1,25(OH)_2D_3$ stimulates the proliferation of more pathogenicidal macrophages ([M_φ] which produce reactive oxygen and nitrogen species; cathelicidin and beta-defensin; and show enhanced chemotaxis) and suppresses the maturation of dendritic cells which favours the differentiation of naïve CD4+ T Cells to anti-inflammatory T-regulatory Cells (Treg). In the adaptive immune system $1,25(OH)_2D_3$ affects naïve CD4+ T Cell differentiation to down-regulate release of pro-inflammatory mediators from type 1 and type 17 T helper cells (Th1 and Th17) and upregulate release of anti-inflammatory mediators from type 2 T helper cells (Th2).



Effect of vitamin D on adaptive immune responses.

In the adaptive immune system, $1,25(OH)_2D_3$ has been found to potentiate T cell activation *via* the classical T cell receptor signalling pathway (82), and skew the phenotype of CD4+ T helper cell populations from pro-inflammatory mediator releasing Th1/Th17 cells towards anti-inflammatory mediator releasing Treg/Th2 cells (depicted by the orange area in Table 1.3), thus decreasing the production of pro-inflammatory cytokines IL-2, IFN-γ, IL-21 and IL-17 (83). By down-regulating the pro-inflammatory Th1/Th17 response and up-regulating an anti-inflammatory Th2/Treg response via induction of IL-10 secretion, vitamin D's actions may prevent inflammatory damage during an overzealous response to respiratory pathogens. It can be argued, however, that this action may represent a double-edged sword, as it may hinder adaptive immunity and increase susceptibility to infection. A clinical trial of vitamin D supplementation for the prevention of congestive heart failure offers *in vivo* findings in human subjects that support the aforementioned work conducted *in vitro* and in murine models which suggest vitamin D stimulates anti-inflammatory conditions: this study found that members of the intervention arm who received 2,000 IU per day of cholecalciferol for nine months had significantly higher serum concentrations of the anti-inflammatory cytokine, IL-10, than members of the placebo arm, and significantly lower serum concentrations of the pro-inflammatory cytokine, TNF-α (84).

1.2.4. Immune dysregulation in older adults.

Older adults are at increased risk of ARI than younger people. Through laboratory work, predominantly in murine models, the following effects of aging on the innate immune response have been discovered: macrophages of aged animals have been shown to experience a 75% decrease in the production of superoxide anions (85-87) and similar reductions have been found in neutrophils (88). Decreasing production of reactive nitrogen species with age has also been documented in macrophages and neutrophils (89, 90). Ageing is also associated with impairment of neutrophil

chemotaxis (91, 92) and antigen presentation by macrophages; the latter phenomenon is associated with a progressive increase in prostaglandin E2 secretion with age, resulting in the inhibition of interleukin-12 (IL-12) secretion and decreased cell surface expression of major histocompatibility complex class II proteins (93). A progressive increase in the production of pro-inflammatory cytokines ("inflammaging") is another characteristic of the ageing immune system, and is believed to be a major contributor to age-related immunopathology (94). Ageing is also associated with attenuation of adaptive immune responses, which may arise due to reduced dendritic cell-mediated stimulation of T and B cells (95). T cell activation has also been found to be impaired by dysregulation of signal transduction proteins in older adults (96, 97). Immunosenescence is also associated with a marked decrease in the population of naïve lymphocytes, caused by reduced T cell thymic output as a consequence of thymic involution (98), decreased progenitor B cell production in bone marrow (99) and accumulation of anergic effector memory CD8+ T cells (100). These defects in adaptive immune responses are associated with decreased protective efficacy of vaccines against respiratory pathogens such as influenza virus (101).

1.2.5. Immune dysregulation in Asthma & COPD patients.

Asthma and COPD sufferers are two further at-risk groups for ARI. The immunopathology of these two respiratory diseases share several similar features, but they differ in their pathogeneses: both diseases are characterised by inflammation of the lower airways and airflow obstruction due to excessive mucus secretion, though airway obstruction in asthma is classically reversible and due to variable inflammation of allergic origin, whereas in COPD inflammation is chronic due to smoking/pollutant-related airway damage and thus the obstruction is non-reversible. Both conditions involve acute episodes marked by a worsening of symptoms, referred to as disease 'exacerbations'. Vitamin D's antimicrobial-stimulating effects in the innate immune system and anti-inflammatory effects in the adaptive immune system suggest a potential beneficial role in the prevention disease exacerbations.

In asthma, the cytokines IL-4, IL-5 and IL-13 are implicated in disease immunopathology, and are believed to predominantly arise from allergen-specific Th2 responses, which lead to B cell proliferation and high levels of IgE antibody production, as well as eosinophil activation and concomitant mucus over-production (102). The 'hygiene hypothesis' was proposed to explain the higher observed incidence of asthma in children who grow up in urban, compared to rural settings, and states this to be due to more sanitary conditions in urban areas causing reduced exposure to pathogens and decreased development of immune regulatory controls at an early age (103). Whilst only in a murine model, recent lab work has shown that perinatal vitamin D deficiency skews the profile of naïve T helper cells towards a Th2 phenotype, along with reduced T reg cell proliferation, and concomitant atopy. Subsequent vitamin D supplementation was found to significantly reduce IgE levels and airway eosinophilia (104). The role of Th17 cells in asthma pathogenesis is also beginning to emerge, challenging the classical view of the disease being predominantly Th2-driven. Non-Th2 cytokine (IL-9, IL-22 and IL-17) expression has been demonstrated in asthma patients (105);increased Th17 cell proliferation has been reported to be characteristic of neutrophilic asthma (106); and *ex vivo*Th17 cell proliferation from $1,25(OH)_2D_3$ –restricted naïve CD4+ T cells has been found to be significantly greater in young asthmatic children compared to healthy controls, and conversely, the inhibition of Th17 cell proliferation was observed when naïve CD4+ T cells were stimulated with $1,25(OH)_2D_3$ (107).

The immunopathology of COPD features an abundance of pulmonary neutrophils and macrophages (108), though despite high levels of these important pathogen-killing innate immune cells, COPD patients remain at high risk of ARI. This has been suggested to be due to defects in immune cell function, for example macrophages of COPD patients show reduced phagocytic capability (109); neutrophils have been found to have impaired chemotaxis (110) and enhanced survival which is responsible for their accumulation (111); and respiratory epithelial cells show impaired mucociliary and barrier function (112), all of which contribute to an impaired innate immune response to

respiratory viruses. Interestingly, expression of the vitamin D receptor (VDR) and major vitamin D activating enzyme (CYP27B1) have been found in airway epithelial cells (113), macrophages (114) and neutrophils (115) which allows vitamin D to exert local autocrine or paracrine actions, and in the case of macrophages, CYP27B1 expression is upregulated with the activation of toll-like receptors (TLR) from viral ligands (116). This therefore raises the question of whether vitamin D's ability to stimulate improved pathogenicity and chemotaxis within these cell types may be of particular benefit to the improvement of innate immune responses in COPD patients, and reduce the risk of exacerbation-causing ARI.

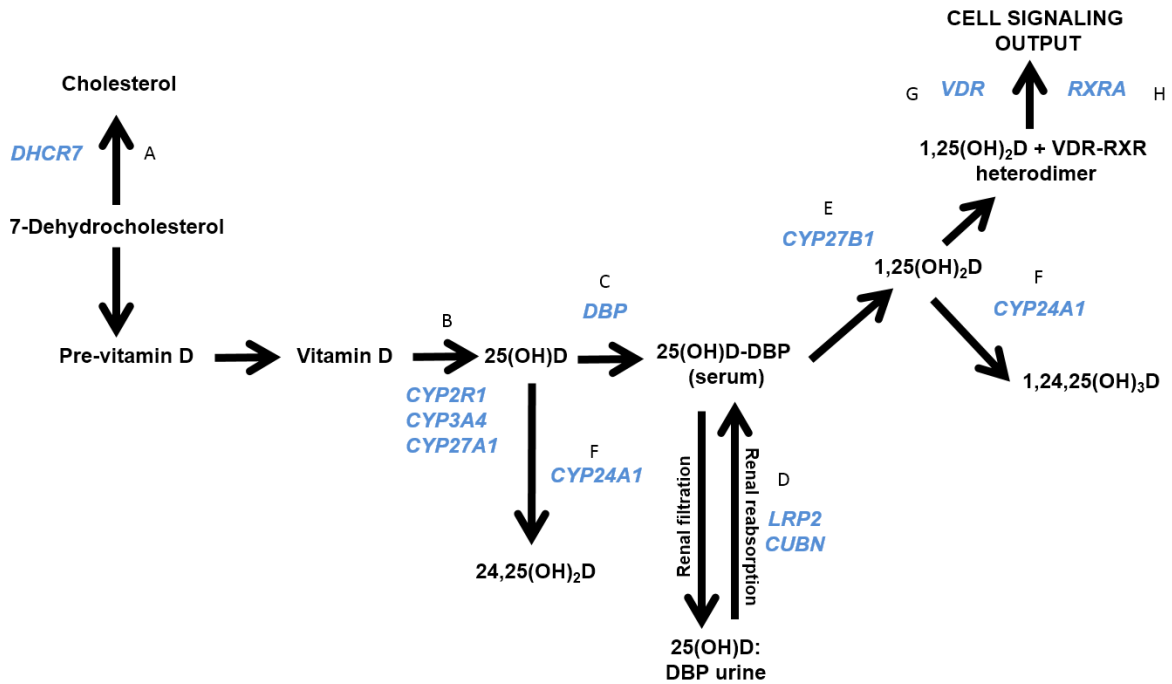
1.2.6. Clinical studies of vitamin D and ARI.

The previously mentioned epidemiological observations of UVR exposure and consequent laboratory studies on vitamin D's immunomodulatory actions have in the past decade prompted a significant number of clinical studies investigating vitamin D in relation to ARI, though no reviews of the literature existed when I commenced my PhD project. To analyse this large body of research I conducted a systematic review during my first year, which was published in the Journal of Steroid Biochemistry and Molecular Biology in December of 2012. I identified a total of 39 clinical studies (4 cross-sectional, 8 case-control, 13 cohort, and 14 clinical trials). 19/25 observational studies reported a significant association between vitamin D deficiency and increased risk of ARI, whilst only 7/14 clinical trials found vitamin D to offer protection against ARI. I present the complete synthesis of these studies and a systematic review of comparable clinical trials in Chapter 3.

1.3. Vitamin D Pathway Genes.

Complex pathways of vitamin D metabolism, transport, and signaling are governed by genes encoding a set of enzyme, carrier, and receptor proteins which in concert achieve the activation of vitamin D from its inert sterol-based precursor; its delivery to target tissues; and its utilisation in a range of cell signaling outputs.

Figure 1.4. A diagram depicting vitamin D metabolic and signalling pathways and genes encoding key players (in blue): DHCR7 (A) encodes the 7-dehydrocholesterol reductase enzyme, which catalyses the conversion of 7-dehydrocholesterol to cholesterol; CYP2R1, CYP3A4, and CYP27A1 (B) encode 25-hydroxylating cytochrome P450 enzymes; the vitamin D binding protein gene (DBP, [C]) encodes the principle vitamin D transport protein; LRP2 & CUBN (D) encode the proteins megalin and cubilin, respectively, involved in renal re-absorption of 25(OH)D via receptor-mediated endocytosis; CYP27B1 (E) encodes the cytochrome P450 enzyme which 1-alpha-hydroxylates 25(OH)D to form 1,25(OH)$_2$D; CYP24A1 (F) encodes the cytochrome P450 enzyme responsible for 24-hydroxylating vitamin D metabolites including 25(OH)D and 1,25(OH)$_2$D; VDR (G) encodes the vitamin D receptor, which binds 1,25(OH)$_2$D and forms a heterodimer with the gene product of RXRA (H) – the retinoid X receptor – to mediate the biological actions of vitamin D.



1.3.1. Metabolism.

Enzymes of the cytochrome P450 family are primarily responsible for the metabolism of vitamin D, however one important non-cytochrome P450 enzyme, 7-dehydrocholestrol reductase (DHCR7) is involved as it converts the vitamin D pre-cursor, 7-dehydrocholesterol (7DHC) to cholesterol by removal of the C (7-8) double bond in the sterol's B ring (117); a reaction that removes 7DHC from the vitamin D synthetic pathway. Thus the balance of vitamin D or cholesterol production is determined to some degree by the level of photo-activation vs. reduction of 7DHC. *DHCR7* gene (pathway A on figure.4) is located on chromosome 11q13.4, spans 24.7 kilo-base pairs (kbp), contains 9 exons and encodes the 475 amino acid, 54.5 kilodalton (kDa) DHCR7 protein. Over 40 single nucleotide polymorphisms (SNP) of *DHCR7* have previously been implicated in the pathogenesis of Smith–Lemli–Opitz Syndrome (SLOS) (118): an autosomal recessive disease which causes multiple congenital deformities due to impeded cholesterol synthesis (119). Interestingly, reports of recurrent ARI (otitis media and pneumonia) have been made in the SLOS patients (120, 121), which may arise due to dysregulation in the vitamin D pathway. One SNP (rs12785878) upstream of the *DHCR7* gene has also been identified in a recent genome wide association study (GWAS) in association with vitamin D status (122): homozygous carriers of the rare allele were found to have lower associated serum 25(OH)D concentration, a finding which has been replicated in other studies (123, 124). Colleagues within our centre have also recently shown that an extended haplotype in *DHCR7* is under positive selection in populations living in Northern latitudes, and suggest this to be a mechanism which offered protection from vitamin D deficiency to humans when we migrated away from the equator; towards conditions of scarcer UVB radiation (125).

The first enzymatic step in vitamin D metabolism is the 25-hydroxylation of cholecalciferol (pre-vitamin D) to 25(OH)D. At least six enzymes belonging to the cytochrome P450 superfamily of monoxygenases have been found to possess 25-hydroxylation activity in the human liver: CYP2D25, CYP2J1, CYP2C11, CYP3A4, CYP27A1, and CYP2R1, but which of these enzymes are involved in the 25-

hydroxylation of vitamin D was unknown until the turn of the 21st century when several studies reported findings that CYP27A1, CYP2R1, and CYP3A4 have vitamin D metabolic activity (126-128). *CYP2R1* gene (pathway B on figure.4) spans 14.2 kbp on chromosome 11p15.2, contains 5 exons and encodes the 501 amino acid, 57.4 kDa microsomal enzyme, CYP2R1 which is now generally believed to be the primary enzyme for 25(OH)D synthesis (52). Several SNP in *CYP2R1* have been found to associate with vitamin D status, both in candidate gene association (CGA) studies and GWAS (122, 124, 129), and one promoter region SNP, rs10766197 has also been found to significantly associate with lung function and IgE concentration in asthmatic cohorts (130, 131), which is an interesting finding in the light of a laboratory study which found perinatal deficiency in 25(OH)D concentration lead to a Th2-skewed T cell phenotype and atopy in offspring (104). Another finding which ties CYP2R1 to immune-related pathology comes from a study which found 20% lower levels of *CYP2R1* expression in patients with nonalcoholic steatohepatitis (NASH) or chronic hepatitis C (CHC), compared to healthy controls (132), and further reports have been made of an association between severe vitamin D deficiency and CHC incidence, or severity (133, 134), supporting the argument for vitamin D's ability to offer protection against viral infection. Lowered expression of CYP27A1 enzyme was also reported to associate with CHC (45% lower in CHC patients vs. healthy controls) (132). This 531 amino acid, 60.2 kDa mitochondrial enzyme is encoded by *CYP27A1* gene (pathway B on figure.4) which spans 33.5 kbp on chromosome 2q35 and contains 9 exons. Over 50 SNP in *CYP27A1* have been found to associate with cerebrotendinous xanthomatosis, a rare disease which occurs due to dysregulated cholesterol and bile acid metabolism (135). The level of CYP27A1-mediated 25-hydroxylation in humans is not currently known, however. It has been demonstrated that CYP2R1 knock-out mice show a 50% decrease in 25(OH)D production, which suggests there's redundancy in the 25-hydroxylase pathway (136), however previous GWAS did not identify a significant association between SNP in *CYP27A1* and circulating vitamin D metabolite concentrations (122, 124), and *CYP27A1*-knockout mice do not display the clinical features of rickets (137), therefore it has been questioned whether CYP27A1 is involved in the 25-hydroxylation of vitamin D at physiologically-relevant concentrations. CYP3A

subtypes are the most abundant hepatic cytochrome P450 enzymes and are capable of metabolising roughly half of all currently available prescription drugs (138). The non-specific, 57.3 kDa, microsomal CYP3A4 enzyme comprises503 amino acids, encoded by *CYP3A4* gene (pathway B on figure4) which spans 27.3 kbp, containing 13 exons, located on chromosome 7q21.1. It's expressed in both the liver and small intestine and has been demonstrated to be more efficient in 24- and 25-hydroxylation of vitamin D_2, than vitamin D_3 (53, 127). Interestingly, 1α,25$(OH)_2D_3$ has also been shown to stimulate CYP3A4 expression *in vitro,* in a range of cell types (139-145), thus vitamin D supplementation may be an effective adjunct to drug therapies which rely upon CYP3A4 for first pass metabolism.

After vitamin D has been 25-hydroxylated to its intermediate metabolite it is then activated by the 56.5 kDa,1α-hydroxylating CYP27B1 enzyme comprising 508 amino acids; encoded by *CYP27B1* gene (pathway E, figure 4) which spans 6.7 kbp, with 9 exons, located on chromosome 12q14.1. Renal synthesis of 1α,25$(OH)_2D_3$ was reported over 40 years ago (146), but more recent reports of extra-renal synthesis have emerged, and it is now widely accepted that localized expression of CYP27B1 and subsequent production of 1α,25$(OH)_2D_3$ allows vitamin D to act in a autocrine, or paracrine fashion to affect a range of important immune cell types, including monocytes (147), macrophages(148), dendritic cells(149), and respiratory epithelial cells (113). SNP in *CYP27B1* gene have been found to associate with circulating 1α,25$(OH)_2D_3$ concentration and susceptibility for several immune-related diseases: in a cohort of chronic hepatitis C patients, CC genotype for a promoter region SNP (rs10877012) associated with a 12 pmol/ml lower serum 1α,25$(OH)_2D_3$ concentration and a 35% decrease in sustained virologic response rate, compared to those with the AA genotype(133). Heterozygosity for the same SNP has also been found to associate with increased incidence of Hashimoto's thyroiditis and Graves' disease (150), and another SNP (rs703842) in near complete linkage disequilibrium (LD) with rs10877012 has been identified by GWAS to associate with Multiple Sclerosis (151, 152). The regulation of *CYP27B1* in the kidney is under strict control by calcium and phosphate homeostatic systems, whereby parathyroid hormone (PTH), and low Ca^{2+} and PO_4^{3-}

concentrations stimulate *CYP27B1* expression, then 1α,25$(OH)_2D_3$ limits its own synthesis via VDR-mediated down-regulation of *CYP27B1*, however in extrarenal cells there exists different mechanisms of *CYP27B1* expression, for example it has recently been demonstrated that in human macrophages, surface toll-like receptors (TLRs) can be activated by *M.tuberculosis* to stimulate the expression of *CYP27B1* and *VDR* and which allows activated vitamin D to stimulate production of the anti-microbial peptide, cathelicidin which is a potent bactericidal compound for this pathogen (116).

In the kidney, the negative feedback loop which down-regulates *CYP27B1* expression, seemingly to ensure active metabolite concentration does not rise too high, is helped by VDR-mediated up-regulation of *CYP24A1* gene (pathway F, figure 4). The gene spans 20.5 kbp, contains 12 exons, is located on chromosome 20q13 and encodes the major vitamin D catabolic enzyme, CYP24A1 (153) which is comprised of 514 amino acids, with a molecular weight of 58.9 kDa. Under normal conditions *CYP24A1* is a highly inducible gene, capable of causing a 20,000-fold increase in local enzyme levels in response to conditions of high 1α,25$(OH)_2D_3$ concentration (154), thus CYP24A1 enzyme largely depicts the bio-availability of vitamin D: *in-vitro* work has shown that CYP24A1-null mice have a 10-fold longer 1α,25$(OH)_2D_3$ half-life (roughly 60 vs. 6 hours) (155), and CYP24A1 is key to the regulation of vitamin D synthesis: in macrophages it has been proposed that hypercalcaemic conditions arise due to an alternative splicing variant of *CYP24A1* that generates a catalytically dysfunctional enzyme (156), and in renal epithelial cells, loss of function mutations in *CYP24A1* have recently been linked to the development of nephrolithiasis and nephrocalcinosis due to hypervitaminosis D (157).

Serum 25(OH)D concentration is not only impacted by 25-hydroxylating enzymes; two important receptors in the vitamin D pathway are required for efficient renal re-uptake of carrier-bound vitamin D: low density lipoprotein receptor-related protein-2 (LRP2, also known as megalin),is a large (4655 amino acid, 522 kDa) cell-surface receptor protein believed to be required for cell uptake of vitamin D

by endocytosis, and is encoded by the *LRP2* gene (pathway D, figure 4) which spans 235.6 kbp on chromosome 2q31.1 and contains 79 exons. More than 10 mutations in *LRP2* gene have been found to associate with Donnai-Barrow Syndrome (DBS), characterised by diaphragmatic hernia, developmental delays, and ocular; sensineural; and craniofacial abnormalities (158). As LRP2 is a non-selective multi-ligand receptor expressed in the neuroepithelium, as well as renal proximal tubule cells, DBS most likely occurs due to the loss of cell uptake for a range of macromolecules, including proteases, protease inhibitors and lipoproteins (159, 160). Interestingly, whilst *LRP2*-knockout mice develop vitamin D deficiency (161) there are currently no *in-vivo* reports of *LRP2* mutation in association with vitamin D status, which suggests the renal re-uptake pathway differs in humans, or there is simply a lack of studies which have investigated this gene in relation to vitamin D metabolism. The second cell-surface receptor involved in vitamin D metabolism is the 3623 amino acid, 398.7 kDa, intrinsic factor-cobalamin receptor (cubilin), which like megalin is expressed in renal proximal tubules and can facilitate the endocytosis of carrier-bound vitamin D. Cubilin is encoded by *CUBN* gene (pathway D, figure 4) which spans 305.9 kbp on chromosome 10p12.31 and has 67 exons. Unlike megalin, murine *CUBN*-knockouts do not display vitamin D deficiency, however loss of *CUBN* function has been linked to a significant increase in urine and decrease in serum 25(OH)D concentration in both dogs (162) and humans. The latter has been found in sufferers of Imerslund-Gräsbeck syndrome (IGS), a rare autosomal recessive disorder caused by vitamin B_{12} deficiency, which strongly associates with several *CUBN* mutations (163).

1.3.2. Transport.

To avoid excess tissue dispersion and urinary excretion, lipophilic vitamin D parent compound and metabolites primarily circulate in the blood bound to their carrier protein, vitamin D binding protein (DBP, also known as Group-specific Component [GC]). Thus DBP ensures effective delivery of vitamin D to target tissue for metabolism (164) and provides a reservoir for metabolised vitamin D to prevent

deficiency in times of reduced synthesis or dietary intake (165). The *DBP* gene (pathway C, figure 4) spans 7.4 kbp on chromosome 19q13.3, has 13 exons and encodes a 474 amino acid, 53 kDa protein. It is a highly polymorphic gene: over 120 variants are known to arise (166), though 2 SNP in exon 11 (rs4588, T>G/Asp>Glu and rs7041, C>A/Thr>Lys) give rise to 3 common DBP isoforms: group-specific component 1 fast (Gc1-F), Gc1-slow (Gc1-S), and Gc2, the latter isoform has been reported to show lower affinity for 25(OH)D than the Gc-1 isoforms (167). *DBP* gene variants associate with vitamin D levels more so than other vitamin D pathway genes: GWAS and CGAS have identified at least 9 SNP which associate with circulating 25(OH)D concentration (122, 124, 168-172), though they only explain around 5% of variation in total 25(OH)D level. The precise level of carrier binding vitamin D undergoes and what effect this may have on vitamin D's actions is a topic of much debate, but it is generally accepted that around 85% of 25(OH)D in circulation is bound to DBP, with the remaining 15% comprising a 'bioavailable' portion which is either bound to lower affinity serum albumin proteins or unbound, the latter being only around 0.1% of total 25(OH)D (173). The free-hormone hypothesis states that only the unbound portion of a hormone is biologically active, and is maintained in dynamic equilibrium with the bound portion, which if true challenges the wisdom of taking total serum 25(OH)D concentration as a measure of vitamin D status. Furthermore, it has been proposed that the portion of unbound 25(OH)D is of particular importance to vitamin D's extra-renal actions as DBP carriage limits cell uptake and tissue-specific 1α-hydroxylation (174). DBP has also been found to form a dimer with macrophage activating factor (MAF), whereby DBP-MAF stimulates activation of macrophages with increased superoxide formation and enhanced phagocytic capacity, but this also drives up airway inflammation (175). Interestingly, Gc-2 genotype associates with decreased DBP-MAF dimerization (176), therefore *DBP* genotype may influence immune responses independent of vitamin D. Several observational studies have reported a significantly greater frequency of Gc-1f isoform in COPD diagnosis, suggesting high-affinity DBP genotype to be a risk factor for disease (177-180), though these associations were predominantly in Asian populations; a recently conducted meta-analysis of the 12 case-control studies which have investigated DBP genotype on the risk of COPD found there to

be no overall effect when controlling for ethnicity (181). Studies investigating DBP genotype in relation to asthma are much fewer, though one case-control study of a Chinese han cohort found Gc-2 genotype to associate with increased risk of disease (182), and another found a mild association between rs7041 and total serum IgE concentration in Caucasians (131). Findings have also been reported in the context of other infection related conditions: Gc-2 carriage was 2-fold higher in rheumatoid fever patients than healthy controls in a study conducted in Kuwait (183), and homozygosity for Gc-2 was significantly higher in active tuberculosis patients than healthy controls in Brazilian Gujarati Asians, but not South African participants (184).

1.3.3. Signalling.

The vitamin D signalling pathway utilises the supply of vitamin D generated and maintained by metabolic and transport pathways. Signalling is mediated via the vitamin D receptor (VDR), a 427 amino acid, 48.3 kDa nuclear phosphoprotein which binds $1\alpha,25(OH)_2D_3$ with high affinity. VDR is encoded by *VDR* gene (pathway G, figure 4) which spans 101.5 kbp on chromosome 12q13.11, and contains 11 exons. VDR-mediated control of gene expression is global: VDR is basally expressed in almost every cell type in the body; highly expressed in metabolic tissues such as the skin, kidneys, intestine and thyroid glands (185, 186), and there are an estimated 100-500 $1\alpha,25(OH)_2D_3$ target genes per tissue type throughout the body (187). Over 600 polymorphisms in *VDR* have been identified (188), and owed to the gene's two promoter regions and six untranslated exons (189) a large quantity of tissue-specific VDR transcripts are expressed. However 4 SNP, named after the restriction endonuclease used to identify them, have been suggested to give rise to 4 functionally distinct VDR isoforms, and have been the focus of research in a wide range of disease contexts: *FokI* is a non-synonymous mutation (rs10735810/rs2228570, C[F]>T[f]) that alters the gene's 5' start codon position in exon 2, creating a VDR protein 3 amino acids longer in homozygotes for T allele, which has been proposed to alter VDR transactivation (190, 191). *TaqI* (rs731236, T>C) occurs at codon 352 of exon 9

and is a synonymous mutation; *ApaI* (rs7975232, A>C) and *BsmI* (rs1544410, A>G) are silent mutations which occur in intron 8. *TaqI, ApaI* and *BsmI* are in high LD with a poly(A) microsatellite length polymorphism located in the gene's 3' untranslated region (UTR) which has a variable number of tandem repeats (VNTR), resulting in alleles with long, or short poly(A) stretches (192). As the poly(A) length polymorphism occurs in a gene region which exerts a strong influence on mRNA half-life (193), and due to its high LD relationship with *TaqI, ApaI* and *BsmI,* it has not yet been established which loci, if any, has a functional effect. There have however been many studies which report findings that link *VDR* gene function with asthma and COPD: laboratory studies have shown that *VDR* wild-type mice can be induced to develop experimental allergic asthma, with characteristic airway hyperresponsiveness, high IgE levels, eosinophilia and heighted Th2 cytokine levels, however *VDR*-knockout mice do not develop the condition despite showing elevated IgE and Th2 cytokine concentrations (194). A case-control study on a pediatric, bronchial asthmatic cohort in Egypt found higher IgE levels in atopic asthmatic children with the TT (ff) genotype for *foci* (χ^2 9.9, P=0.007).They also report a lower frequency of CC (FF) genotype in asthmatic children vs. healthy controls (OR 0.23, 95% CI: 0.07 – 0.78, P=0.02); and a lower still frequency in atopic asthmatic children vs. healthy controls (OR 0.11, 95% CI: 0.02 – 0.46, P=0.002) (195). Variation in IgE levels for *fokI* genotype were also found in a case-control study of young, African Americans in the U.S (P<0.001), as was variation in baseline spirometry values (P<0.05), and allergen skin test results (P=0.003) (130). A case-control study in adult, Chinese Hans report a higher frequency of *ApaI*C allele in asthmatic participants vs. healthy controls, suggesting this allele confers increased risk of asthma (OR 1.33, 95% CI: 1.10–1.60, P=0.009) (196). *ApaI* and *BsmI* mutations were found to associate with asthma incidence and atopy in a family-based cohort of French-Canadians in Quebec, where *ApaI* C allele was over-transmitted to offspring, again suggesting an increased risk of asthma (P<0.05) (197). A higher frequency of *ApaI* C allele in asthmatics, compared to healthy controls were also observed in a nested case-control study of participants from the Nurses' Health Study (NHS), however this was a validation cohort or the family-based Childhood Asthma Management Program (CAMP) study which found under-transmission

of the *ApaI* C allele to offspring, suggesting C allele confers protection against asthma(198). Homozygosity for the rare allele of *ApaI* was found to associate with better asthma control test score (Global Initiative for Asthma guidelines [GINA], P=0.001) and lower daily activity limitation (P=0.004), in a cohort of Greek children (199).

Currently, very little research has been conducted on *VDR* variants in the context of COPD. One group has investigated them in relation to quadriceps strength (a complication of the disease), and found homozygosity for the C allele of *FokI* to associate with lower quadriceps strength in both cases and controls, and the *b* allele for *BsmI* to associate with increased quadriceps strength in cases, compared to controls (200). Another group has investigated genotype for *FokI, TaqI*, and *BsmI* polymorphisms on the incidence of COPD exacerbations for patients classified as frequent vs. non-frequent exacerbators, and found no significant relationship (201), however *VDR* variants have been found to associate with acute respiratory infection and tuberculosis (TB): a 7-fold greater adjusted relative odds for *ff* genotype in *FokI* for children hospitalized for LRI (predominantly RSV bronchiolitis), compared to healthy controls has been reported (202), as has a 2-fold greater odds of *ff* genotype for *FokI* in spinal TB patients, compared to healthy controls in a Chinese Han cohort (203). Transmission of the 4 common *VDR* polymorphisms was investigated in a large family-based case-control study of TB patients in Guinea, Gambia, and Guinea-Bissau. They found significant over-transmission of FA haplotype for *FokI-ApaI* in TB cases (χ^2 11.6, P=0.0007), but individual *VDR* SNP did not associate with the risk of TB(204).A large number of case-control studies have investigated a link between *TaqI* and TB, with conflicting results: A recent meta-analysis of 21 studies, pooling 2,960 TB cases and 3,894 healthy controls found no overall effect of *TaqI* polymorphismon risk of TB. However, In a randomized control trial of vitamin D supplementation as an adjunctive treatment for UK adults with smear-positive pulmonary TB, *tt* genotype for *TaqI* was found to enhance the effect of vitamin D supplementation on the time to sputum culture conversion (8.09, 95% CI: 1.36-48.01, P=0.02) (205).

The effect of mutations on vitamin D signaling may not be limited to VDR. For optimal signaling, ligated VDR protein binds another receptor protein, the retinoid-X receptor (RXR), to form a heterodimer which causes the release of co-repressor proteins. This stimulates the recruitment of co-activator proteins (219) and improves receptor binding to a vitamin D response element (VDRE) within a gene's promoter region, therefore affecting transcription (220). There are three known isoforms of RXR: α, β, and γ; RXR-α has been most extensively researched in relation to VDR. RXR-α is a 462 amino acid, 50.8 kDa nuclear receptor protein encoded by the 123.5 kbp *RXRA* gene (pathway H on figure 4) located on chromosome 9q34.3. Very little research has been conducted on RXR genes in relation to vitamin D and health outcomes, but one study did identify a stepwise increase in circulating $1\alpha,25(OH)_2D_3$ concentration for each A allele of rs9409929 in *RXRA* (P=0.003 for the trend), though this finding became non-significant after controlling for multiple comparison testing (206). Functional mutations of *RXRA* may be of particular importance in the immunopathology of asthma and COPD: RXR-α has been found to regulate the transcription of macrophage cytokines (CCL6 and CCL9) which stimulate leukocyte recruitment to sites of inflammation, consequently impacting the efficacy of innate immune responses (207).

1.4. Summary.

In summary, ARI impose a heavy toll in the form of lost productivity costs due to school and work absenteeism, and healthcare related costs due to GP visits, hospitalisation, and over-the-counter/prescription medications. But most importantly, ARI are responsible for a large loss of human life, which is seen in both developing and developed countries and especially in at-risk groups for ARI, such as the elderly and those with chronic respiratory diseases. People within these at-risk groups are more susceptible to ARI due to defects of the immune system which arise from age-related decline or disease related pathology and can compromise the immune system's capacity to stage effective responses to respiratory pathogens. A large and ever-growing body of *in vitro* research suggests that vitamin D modulates immune responses by stimulating enhanced bactericidal, virucidal, and fungicidal properties within immune cells that are active during response to respiratory pathogens. To a large extent *in vivo* research has corroborated these findings by illustrating an observational association between vitamin D deficiency and increased risk of ARI, and protection against ARI from vitamin D supplementation has been demonstrated in some clinical trials. Other clinical trials have been null however, and in a few instances supplementation has increased the risk of ARI. The mixed results we have seen may be due to important methodological inconsistencies or currently uncontrolled-for effect modifiers. Currently, no clinical trial of vitamin D in the prevention of ARI has investigated the effect of variation in genes which encode proteins involved in vitamin D metabolism, transport, and signalling, and specifically in at-risk populations for ARI. Mutations within these genes may result in aberrant or under-/over-expressed proteins which are integral to vitamin D's immunomodulatory effects. Thus, genetic variation has the potential to impact an individual's normal vitamin D status; their response to vitamin D supplementation; their utilisation of available active vitamin D metabolite, and consequently their susceptibility to ARI.

1.5. Hypothesis and Objectives of the project.

This PhD project centred around three clinical trials of vitamin D supplementation to prevent ARI in asthma patients, COPD patients, or healthy older adults. The hypothesis was that in these at-risk populations for ARI, genetic polymorphisms affecting pathways of vitamin D metabolism, transportation, or signalling would:

A.) Associate with serum concentrations of 25-hydroxyvitamin D;

B.) Associate with clinical markers of asthma and COPD phenotype;

C.) Associate with risk of acute respiratory infection, or exacerbations of asthma or COPD, independent of vitamin D status;

D.) Modify the effect of vitamin D supplementation in the prevention of ARI / exacerbations of asthma or COPD; If true will:

   i. Associate with pro-inflammatory mediators released during immune response to respiratory pathogens;

   ii. Associate with the level of expression of the gene in which the SNP arises.

In order to test these hypotheses I addressed the following objectives:

A.) To perform a systematic review of clinical studies which have investigated a link between vitamin D and the incidence/prevention of ARI, or exacerbations of asthma and COPD, then to follow this up with a meta-analysis of randomised controlled trials in order to determine the overall protective effect of vitamin D, and to quantify the level of heterogeneity that exists between these studies.

B.) To identify from existing literature, candidate single nucleotide polymorphisms in vitamin D metabolism, transport, and signalling pathways which may associate with vitamin D status; clinical markers of asthma/COPD phenotype; ARI; and modify the effect of vitamin D supplementation.

C.) To extract DNA from whole blood samples then develop and perform assays to genotype participants for the candidate SNPs described above, and to assay concentrations of 25-hydroxyvitamin D and immunological parameters in serum.

D.) To conduct cross-sectional analyses in three separate clinical trial cohorts to determine the level of variation in baseline vitamin D status explained by environmental and genetic factors, and whether vitamin D status and/or genetic factors associate with clinical features of asthma/COPD.

E.) To conduct statistical analyses to determine whether candidate SNP:

i. Associate with risk of ARI, or exacerbations of asthma or COPD, independent of vitamin D status.

ii. Modify the effect of vitamin D supplementation on risk of ARI, or exacerbations of asthma or COPD.

iii. Associate with pro-inflammatory mediators released during immune response to respiratory pathogens.

iv. Associate with the level of gene expression in which the SNP arises.

2. Methods.

2.1. Systematic review of vitamin D and ARI studies.

This section describes the search and selection method used in a systematic review I conducted of clinical studies which have investigated vitamin D in prevention of ARI, which is presented in chapter 3.

2.1.1. Search method.

The PubMed database was searched on 17th October 2012 using the terms 'vitamin D' and 'respiratory infection'. No restrictions were placed on language of publication or on the age, sex, ethnic origin, baseline vitamin D status or presence or absence of comorbidity in populations studied.

Studies were classified into one of three categories: potentially eligible primary studies, relevant review articles and ineligible primary studies. Full text of potentially eligible primary studies was reviewed to confirm eligibility according to criteria presented below. Relevant review articles were retrieved and screened for additional primary studies. All articles were assessed for eligibility by one author (DAJ) then re-assessed by a second (ARM).

2.1.2. Exclusion/Inclusion Criteria.

Studies were screened by title and abstract to evaluate whether they met the following eligibility criteria:

Inclusion criterion:

- Cross-sectional studies, case-control studies, cohort studies or clinical trials conducted in human subjects, and investigating the relationship between serum concentration of vitamin D metabolites or clinical manifestations of vitamin D deficiency, or effect of dietary intake or administration of vitamin D or its analogues, on risk of acute respiratory infection or acute exacerbation of asthma or Chronic Obstructive Pulmonary Disease (COPD).

Exclusion criterion:

- Studies relating exclusively to tuberculosis (these are reviewed elsewhere (208)), and were beyond the remit of this review as tuberculosis is classically regarded as a chronic respiratory tract infection, with symptom duration usually exceeding 30 days).

2.2. Clinical trials.

All analyses conducted in support of this thesis were on data which arose from three randomised, double-blind, placebo-controlled trials of vitamin D supplementation in prevention of ARI or disease exacerbations, in patients with asthma (ViDiAs trial (209)), COPD (ViDiCO trial (210)), or healthy older adults and their caregivers living in sheltered accommodation (ViDiFlu trial (209)).

2.2.1. Participants.

ViDiAs trial.

Adult patients with a medical record diagnosis of asthma treated with inhaled corticosteroids (ICS) were identified by searching medical databases at 60 general practices and asthma clinics in 2 Acute

National Health Service Trusts in London, UK. From this search 297 prospective participants were invited for screening which took place between 27th August 2009 and 25th June 2012.

Patients were deemed eligible if they:

- were between 16 and 80 years of age;
- had a medical diagnosis of asthma treated at BTS (British Thoracic Society) Step 2 (taking inhaled corticosteroids daily (211)) or above;
- showed either a ≥12% increase in forced expiratory volume in one second (FEV1) after inhalation of 400µg of salbutamol, or a ≥20% diurnal variability peak in expiratory flow (PEFR), or methacholine PC20 (concentration of methacholine causing a 20% fall in FEV1) < 8g/L.

In addition to this, patients agreed to the required visits, were contactable by telephone, were able to give written informed consent to participate and, if a woman of child-bearing potential, agreed to use a reliable form of contraception for the duration of the study.

Patients were excluded if they:

- had a diagnosis of COPD;
- had known sarcoidosis, hyperparathyroidism, nephrolithiasis, active tuberculosis, vitamin D intolerance, liver failure, renal failure or any malignancy not in remission for at least 3 years;
- were taking dietary supplements containing >10µg per day of vitamin D;
- had a baseline corrected serum calcium >2.65mmol/L;
- had a baseline serum creatinine > 125µmol/L;
- had a smoking history > 15 pack years (calculated by the number of packs of cigarettes smoked per day divided by 20, and multiplied by the number of years the person has smoked for);

- were breastfeeding, pregnant or planning a pregnancy, undergoing treatment with any other IMP or device up to 4 months before randomisation onto this trial;
- were taking a cardiac glycoside (e.g. Digoxin), carbamazepine, phenobarbital, phenytoin, primidone or benzothiadiazine derivatives at a dose higher than recommended in the British National Formulary (BNF) or in combination with a calcium supplement.

Of the 297 screened respondents, 37 were ineligible to participate; 10 were eligible but declined, leaving 250 successfully recruited and randomised patients, who were given 6 x 2-monthly doses of 6ml of active IMP (containing 3mg of Vitamin D3, equating to 120,000 IU) or placebo over the period of one year. The study was approved by East London and The City Research Ethics Committee 1 (ref 09/H0703/67) and written informed consent was obtained from all participants before enrolment.

ViDiCO trial.

Adult patients with a medical record diagnosis of COPD, emphysema or chronic bronchitis were identified by database searches at 60 general practices and COPD clinics in 4 Acute National Health Service Trusts in London, UK. From this search 320 prospective participants agreed to attend screening which took place between 11th September 2009 and 12th April 2012.

Patients were deemed eligible if they:

- were over 40 years old;
- had a medical diagnosis of COPD, emphysema or bronchitis;
- had a post-bronchodilator ratio of forced expiratory volume in one second to forced vital capacity (FEV1/FVC) < 70%, or post-bronchodilator FEV1/slow VC < 70%;
- had a smoking history ≥ 15 pack years;

In addition to this, patients agreed to the required visits, were contactable by telephone, were able to give written informed consent to participate and, if a woman of child-bearing potential, agreed to use a reliable form of contraception for the duration of the study.

Patients were excluded if they:

- had a current diagnosis of asthma;
- had known clinically significant bronchiectasis, sarcoidosis, hyperparathyroidism, nephrolithiasis, active tuberculosis, vitamin D intolerance, liver failure, renal failure or any malignancy not in remission for at least 3 years;
- were taking dietary supplements containing >10µg per day of vitamin D;
- had a baseline corrected serum calcium >2.65mmol/L;
- had a baseline serum creatinine > 125µmol/L;
- required long-term oxygen therapy for ≥ 12 hours per day;
- were breastfeeding, pregnant or planning a pregnancy;
- were undergoing treatment with any other IMP or device up to 4 months before randomisation onto this trial;
- were taking a cardiac glycoside (e.g. Digoxin), carbamazepine, phenobarbital, phenytoin, primidone or benzothiadiazine derivatives at a dose higher than recommended in the British National Formulary (BNF) or in combination with a calcium supplement.

Of the 320 screened respondents, 240 patients were successfully recruited and randomised to receive 6 x 2-monthly doses of 6ml of active IMP (containing 3mg of Vitamin D3, equating to 120,000 IU) or placebo over a period of one year. The study was approved by East London and The City Research

Ethics Committee 1 (ref 09/H0703/67) and written informed consent was obtained from all participants before enrolment.

ViDiFlu trial.

Units, defined as sheltered accommodation schemes, residential care homes, nursing homes, day centres or groups for older adults, in the Greater London area were identified by searching an online directory (www.housingcare.org). Units offering care exclusively for residents with dementia, learning disability, mental health problems, and alcohol/drug dependency were deemed ineligible. The housing associations of eligible units were approached for permission to conduct the trial on their premises. Invitation letters were sent to the residents and carers of the identified units, inviting them to attend a screening visit. Participants were screened between 29th March 2010 to 16th March 2012 and deemed eligible if they:

- were a permanent resident, attendee, staff member or carer at a participating unit;
- were aged 16 or over.

In addition to this, patients agreed to the required visits, were able to give written informed consent to participate and, if a woman of child-bearing potential, agreed to use a reliable form of contraception for the duration of the study.

Patients were excluded if they:

- had a current diagnosis of asthma or COPD;
- had a chronic respiratory infection or chronic cough;
- had any condition requiring treatment with a vitamin D dose of > 10µg per day;

- had known sarcoidosis, hyperparathyroidism, nephrolithiasis, active tuberculosis, vitamin D intolerance, liver failure, renal failure or any malignancy not in remission other than cutaneous basal/squamous cell carcinoma;
- were taking dietary supplements containing >10µg per day of vitamin D;
- had a baseline corrected serum calcium >2.65mmol/L;
- had a baseline serum creatinine > 125µmol/L;
- were unable to complete the symptom diary;
- were breastfeeding, pregnant or planning a pregnancy;
- were undergoing treatment with any other IMP or device up to 4 months before randomisation onto this trial;
- were taking a cardiac glycoside (e.g. Digoxin), carbamazepine, phenobarbital, phenytoin, primidone, long-term immunosuppressant therapy, or benzothiadiazine derivatives at a dose higher than recommended in the British National Formulary (BNF) or in combination with a calcium supplement.

Two hundred and forty successfully recruited and randomised participants had various dosing regimens depending on their status as resident or staff/carer (Table 2.1), as it was deemed unethical to withhold vitamin D supplementation in individuals aged ≥ 65 with limited sunlight exposure. As such, staff and carers were randomised to receive 6 x 2-monthly doses of 6ml of active IMP (containing 3mg of Vitamin D3, denoted PR1) or placebo over the period of one year (denoted PL1), while residents were randomised to receive low-dose vitamin D supplementation (a daily dose of 20µl of active IMP for 1 year, denoted PR3, as well as 6 x 2-monthly doses of 4.8ml of placebo, denoted PL2) or high-dose vitamin D supplementation (a daily dose of 20µl of active IMP for 1 year, denoted PR3, as well as 6 x 2-monthly doses of 4.8ml active IMP containing 2.4mg of Vitamin D3, denoted PR2), thus allowing a comparison between high-dose vitamin D3 and placebo in staff and carers, and between high-dose vitamin D3 and low-dose vitamin D3 in residents and attendees. The study was approved

by East London and The City Research Ethics Committee 1 (ref 09/H0703/112) and written informed consent was obtained from all participants before enrolment.

Table 2.1: ViDiFlu trial dosing regimens.

Participant, arm	IMP regimen received	Vitamin D_3 received	Total vitamin D_3 dose in 1 year	Equivalent daily dose of vitamin D_3
Resident and attendee, control arm	PR3 + PL2	10 micrograms daily	3.65 milligrams	10 micrograms
Resident and attendee, intervention arm	PR2 + PR3	10 micrograms daily + 6 bolus doses of 2.4 milligams	18.05 milligrams	50 micrograms
Staff member and carer, control arm	PL1	Nil	Nil	Nil
Staff member and carer, intervention arm	PR1	6 bolus doses of 3 milligrams	18 milligrams	50 micrograms

** PR1, PR2 and PR3 are Vigantol ® regimens*
*** PL1 and PL2 are Miglyol ® regimens*

Abbreviations: IMP: Investigational medical product, PL1/PR1/PR2/PL2/PR3: Arbitrary identifiers.

2.2.2. Screening Procedures.

All respondents underwent height measurement (using a Seca 220 Telescopic Measuring Rod, Seca, Hamburg, Germany), and weight measurement (using Marsden MMPS-250 column scales, Marsden, Rotherham, UK), and were asked to complete a lifestyle questionnaire detailing age, sex, ethnicity, self-reported Fitzpatrick skin-type (212), self-classified socio-economic position (SEP) using the NS-SEC method (213), daily hours spent outdoors, history of recent sunny holidays abroad (defined as a trip to any location within a latitude 51° North/South of the equator, during the local sunny season, 2 months prior to blood draw, for a duration of ≥1 week), smoking behaviour and consumption of alcohol and supplemental vitamin D. Additionally, for ViDiFlu respondents, researchers recorded the extent of hair loss on the head (Norwood-Hamilton (214) scale for males; Ludwig scale (215) for females), if any, and skin melanin density measured using a DSM II Colormeter (Cortex Technology,

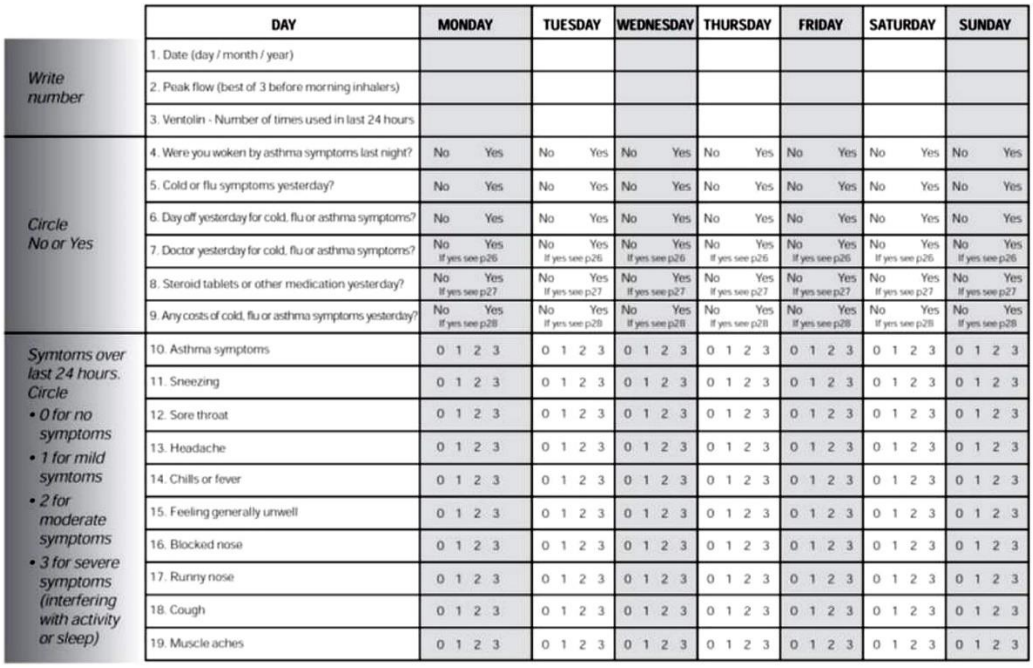
Hadsund, Denmark). ViDiAs and ViDiCO respondents underwent lung function tests: spirometry before and after inhalation of 400 µg salbutamol via a spacer device, performed using a MicroLab ML3500 desktop spirometer (CareFusion GmbH, Hoechberg, Germany) according to American Thoracic Society (ATS) / European Respiratory Society (ERS) recommendations (216), and ViDiAs respondents also completed the asthma control test (ACT) questionnaire (217), and underwent FeNO measurement, performed using a NIOX MINO 09-1100 (Aerocrine, Solna, Sweden) according to ATS / ERS recommendations (218).

All trial respondents were requested to donate a blood sample for subsequent DNA extraction and determination of serum concentration of total 25[OH]D and parathyroid hormone (PTH). A sub-set of 35 ViDiAs, and 44 ViDiCO respondents underwent sputum induction with hypertonic saline, and their samples were processed to make cytospin slides according to methods described by Pizzichini *et al* (219) and detailed in Methods section 2.3.3.

2.2.3. Definition of respiratory outcomes.

Successfully screened participants were given a 2 week run-in period to assess their ability/compliance in completing a daily study diary (Figure 2.1 shows a spread from the ViDiAs symptom diary as an example) to capture signs and symptoms of URI (sneezing, sore throat, headache, subjective sensation of fever or chilliness, malaise, nasal discharge, nasal obstruction, cough), as defined by the Jackson criteria (220); daily severity of asthma/COPD symptoms; daily peak flow readings in the case of ViDiAs trial participants; muscle pain score; use of medication; health care use; work absence; finally, out-of-pocket expenses incurred due to respiratory symptoms or illness.

Figure 2.1. Example of the daily diary used to capture URI symptoms and related measures.



	DAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
Write number	1. Date (day / month / year)							
	2. Peak flow (best of 3 before morning inhalers)							
	3. Ventolin - Number of times used in last 24 hours							
Circle No or Yes	4. Were you woken by asthma symptoms last night?	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes
	5. Cold or flu symptoms yesterday?	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes
	6. Day off yesterday for cold, flu or asthma symptoms?	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes
	7. Doctor yesterday for cold, flu or asthma symptoms?	No Yes If yes see p26	No Yes If yes see p26	No Yes If yes see p26	No Yes If yes see p26	No Yes If yes see p26	No Yes If yes see p26	No Yes If yes see p26
	8. Steroid tablets or other medication yesterday?	No Yes If yes see p27	No Yes If yes see p27	No Yes If yes see p27	No Yes If yes see p27	No Yes If yes see p27	No Yes If yes see p27	No Yes If yes see p27
	9. Any costs of cold, flu or asthma symptoms yesterday?	No Yes If yes see p28	No Yes If yes see p28	No Yes If yes see p28	No Yes If yes see p28	No Yes If yes see p28	No Yes If yes see p28	No Yes If yes see p28
Symtoms over last 24 hours. Circle • 0 for no symptoms • 1 for mild symtoms • 2 for moderate symptoms • 3 for severe symptoms (interfering with activity or sleep)	10. Asthma symptoms	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	11. Sneezing	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	12. Sore throat	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	13. Headache	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	14. Chills or fever	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	15. Feeling generally unwell	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	16. Blocked nose	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	17. Runny nose	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	18. Cough	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	19. Muscle aches	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3

Upper respiratory infections.

Using the captured daily symptom diary scores, URI were defined as influenza-like illness (indicated by presence of cough, fever or chills, and muscle pains) (221), or a cold as defined by the Jackson criteria (220). This method of defining URI has been previously validated by our group using PCR detection of 11 respiratory viruses obtained by nasopharyngeal swabs (222).

Lower respiratory infections.

Daily symptom diary scores were also used to define LRI events in the ViDiFlu trial, according to the Macfarlane criteria, as follows: the five Macfarlane symptoms (cough, sputum production, dyspnoea, wheeze, and chest pain/discomfort) were scored 0-3 by their severity. Presence of cough and at least one other symptom with a score of 1 or more points greater than during the run-in period were used to define an LRI event (223).

Exacerbations of Asthma & COPD.

Disease exacerbations, rather than LRI, were defined from symptom diary data in the ViDiAs and ViDiCO trials, using an algorithm based on the following: for the ViDiAs trial an exacerbation was defined as deterioration in asthma resulting in a) treatment with oral corticosteroids, or b) hospital admission or emergency department treatment, or c) decrease in the morning PEFR to more than 25% below the mean run-in value on 2 or more consecutive days (224). For the ViDiCO trial an exacerbation was defined as a) the occurrence of ≥ 2 major COPD symptoms, or b) the occurrence of 1 major COPD symptom and ≥ 1 minor COPD symptom during ≥ 2 consecutive days. Major symptoms were defined as increase in dyspnoea, sputum volume or sputum purulence as compared with their usual symptoms; minor symptoms were defined as increase in nasal congestion or discharge, wheeze, sore throat, or cough (225).

2.2.4. Investigational medicinal product (IMP).

The IMP administered in all three trials was Vigantol® Oil (Merck Serono, Darmstadt, Germany) – an oily solution containing 0.5mg cholecalciferol (vitamin D3) per millilitre. The placebo was Miglyol® 812 oil (Caesar and Loretz, Hilden, Germany) – an organoleptically identical mixture of palm oil and coconut oil widely used in pharmaceutical practice. Miglyol® 812 oil is the excipient for cholecalciferol in Vigantol® Oil, and is thus identical to Vigantol® Oil in every respect except for the absence of cholecalciferol. Nova Laboratories Ltd (Wigston, UK) produced a computer-generated randomisation sequence and bottled study medication, and treatment allocation was concealed from trial participants and study staff.

2.3. Laboratory analyses.

2.3.1. Biochemistry analysis.

All biochemical analyses for the 3 clinical trials were carried out in the Department of Clinical Biochemistry at Homerton Hospital. This laboratory participates in the international vitamin D external quality assurance scheme (www.deqas.org/). Serum concentrations of 25(OH)D_2 and 25(OH)D_3 were determined by isotope-dilution liquid chromatography–tandem mass spectrometry (226), and summed to give total serum 25(OH)D concentration. Parathyroid hormone (PTH) concentrations were determined using an Architect ci8200 analyser (Abbott Diagnostics, Chicago, IL, USA).

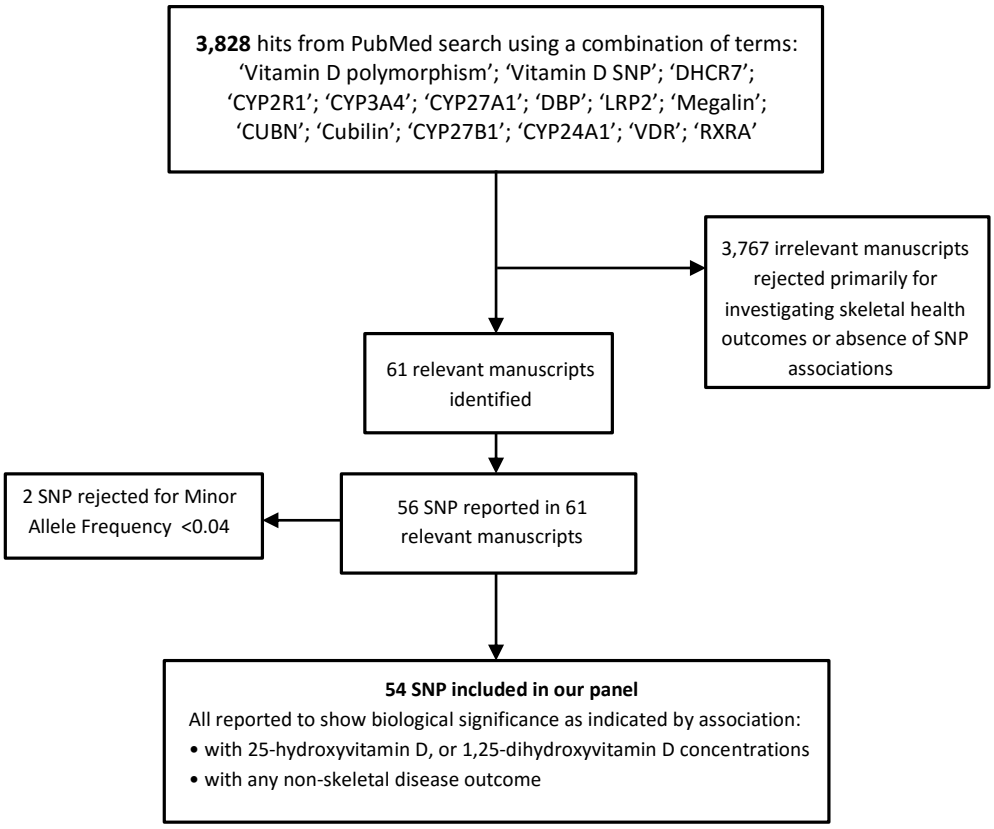
2.3.2. Genotyping.

I selected a panel of 37 single nucleotide polymorphisms and contributed to the genotyping of participants in our three clinical trials, under the supervision of Dr Mimi Hot of Queen Mary University's Genome Centre.

Literature search.

A literature search of the PubMed database was carried out in April of 2012 (figure 2.2), using a combination of the following terms: 'Vitamin D polymorphism'; 'Vitamin D SNP'; 'DHCR7'; 'CYP2R1'; 'CYP3A4'; 'CYP27A1'; 'DBP'; 'LRP2'; 'Megalin'; 'CUBN'; 'Cubilin'; 'CYP27B1'; 'CYP24A1'; 'VDR'; 'RXR'. This search identified 56 single nucleotide polymorphisms (SNP) in 11 vitamin D pathway genes previously reported to associate with circulating concentrations of vitamin D metabolites and/or any non-skeletal disease outcome; 2 SNP were rejected for having a minor allele frequency (MAF) below 4%.

Figure 2.2: A flow diagram depicting the SNP literature search and selection process.



Tag SNP selection.

Twenty-four of the 54 SNP were in high linkage disequilibrium ($r^2 \geq 0.8$) in the HapMap database (release #27: Phase 1, 2 & 3 - merged genotypes & frequencies), thus for these variants, six tag SNP (tSNP) were selected as proxies: this was achieved by retrieving SNP location and allele frequency information ±10 kilo-bases upstream and downstream of all genes, from the Utah residents with Northern and Western European ancestry from the CEPH collection (CEU) which were chosen to represent the majority ethnicity of our clinical trial participants. I then ran a tagging algorithm developed by de Bakker et al (227) via Bioinformatics' Haploview program (v.3.3) (228), selecting the 'pairwise tagging only' option; setting the r^2 threshold to > 0.8; accepting a minimum genotype completeness of 75%; and a MAF threshold of 0.04. Tagging reduced the number of alleles to be genotyped from 54 to 37 SNP, which are listed in Table 2.2.

Table 2.2: Single nucleotide polymorphisms (SNPs) identified as putative modifiers of the effects of vitamin D supplementation.

Gene	Target SNP	Tag SNP [1]	R^2
CYP24A1	rs2762934	-	-
	rs6127118	-	-
	rs2248137	-	-
	rs6013897	-	-
	rs2762939	-	-
CYP27B1	rs4646536	-	-
	rs10877012	rs4646536	1.00
	rs703842	rs4646536	1.00
	rs4646537	-	-
CYP2R1	rs2060793	-	-
	rs10741657	rs2060793	1.00
	rs1993116	rs2060793	1.00
	rs7116978	rs2060793	0.92
	rs10500804	-	-
	rs12794714	rs10500804	1.00
	rs10766197	-	-
CYP3A4	rs2740574	-	-
CYP27A1	rs17470271	-	-
VDR	rs1544410	-	-
	rs731236	-	-
	rs4516035	-	-
	rs4334089	-	-
	rs10783219	-	-
	rs7976091	-	-
	rs11574010	-	-
	rs2853559	-	-
	rs2238136	-	-
	rs7975232	-	-
	rs2228570	-	-
	rs7970314	-	-
	rs11568820	-	-
DBP	rs4588	-	-
	rs2282679	rs4588	1.00
	rs3755967	rs4588	1.00
	rs17467825	rs4588	1.00
	rs1155563	rs4588	0.83
	rs2298850	rs4588	0.95
	rs7041	-	-
	rs222035	rs7041	0.92
	rs842999	rs7041	0.96
	rs2298849	-	-
	rs16846876	-	-
	rs12512631	-	-
	rs2070741	-	-
DHCR7	rs12785878	-	-
	rs4944957	rs12785878	1.00
	rs4945008	rs12785878	0.95
	rs3794060	rs12785878	1.00
	rs7944926	rs12785878	1.00
	rs12800438	rs12785878	1.00
	rs3829251	-	-

Table 2.2 continued.

Gene	Target SNP	Tag SNP [1]	R^2
CUBN	rs3740165	-	-
RXRA	rs9409929	-	-
	rs7861779	-	-
LRP2	rs3755166	-	-

[1] *Six tag SNP were selected to capture 18 SNP, due to a linkage disequilibrium r^2 value of >0.8.*

Abbreviations: SNP: Single nucleotide polymorphism, CYP-: Cytochrome P450-, VDR: Vitamin D receptor, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase, CUBN: Cubilin, RXRA: Retanoid-X receptor-A, LRP2: Low density lipoprotein receptor-related protein-2 (Megalin).

Custom assay design.

Pre-developed assays were available for 34/37 SNP. For the remaining 3 SNP, their FASTA sequences were retrieved from the National Centre for Biotechnology Information's (NCBI) online dbSNP database (www.ncbi.nlm.nih.gov/SNP/) and entered into the Institute for Systems Biology's online Repeat Masker (www.repeatmasker.org) programme, which screens the sequence for repeating bases that may harm the assay's accuracy and masks them. The masked sequence was then used in Applied Biosystems' File Builder application (v3.1) to generate custom assay primer and reporter sequences: rs2740574 in *CYP3A4* (forward primer sequence CCAGGCATAGGTAAAGATCTGTAGGT, reverse primer sequence CTCAAGTGGAGCCATTGGCATA, reporter sequences ACAAGGGCAAGAGAG and ACAAGGGCAGGAGAG), rs3740165 in *CUBN* (forward primer sequence GCAATGAGATTAAATCTTCAGGAAACACA, reverse primer sequence CTGGAGGTATAGGAAGCAG-TGAAG, reporter sequences CCGCCATATGGCCTG and CGCCATACGGCCTG) and rs7861779 in *RXRA* (forward primer sequence TGGCCCATGCACGAGTAG, reverse primer sequence ACCGAGACAGGCCAAACTC, reporter sequences CAGCAGAGGTGGCCGA and CAGCAGAGATGGCCGA).

DNA extraction.

DNA was extracted in Queen Mary University's Genome Centre, using a modified version of Miller et al's salting-out protocol (229) on a Biomek FX robot (Beckman Coulter): 400 uL whole blood was

pipetted into each well of a 2.4ml Elkay plate. 1600 uL water was added to each well and pipetted up and down five times to mix. The plate was then incubated on ice for 2 minutes then centrifuged at 3000 rpm for 10 minutes at 4°C. After centrifugation supernatant was aspirated, then 1600 uL of solution 1 (for 500ml solution: 320ml of 0.5M sucrose; 5ml of 1M tris-CL pH 7.6; 2.5ml of 1M MgCl2; 5ml of 2% sodium azide; 5ml of 100% triton X-100; 162.5ml of water) was added to each well and pipetted up and down 5 times to mix. The plate was then incubated on ice for 2 minutes then centrifuged at 3000 rpm for 10 minutes at 4°C. 1600 uL of supernatant was then aspirated and 1600 uL of solution 1 was added to each well again. Another 2 minutes of incubation on ice and a further round of centrifugation at 3000 rpm, for 10 minutes at 4°C was then carried out. 1800 uL of supernatant was then removed from each well and discarded, then 1100 uL of solution 2 (for 500ml solution: 25ml of 1M tris-Cl pH 8; 40ml of 0.25M EDTA pH 8; 100ml of 10% SDS; 335ml of water) was added to each well, followed by 5.5 uL 20mg/ml proteinase K. The plate was then incubated overnight at 37°C. After overnight incubation, 400 uL of saturated NaCl was added to each well and carefully pipetted up and down 5 times to mix. The plate was then centrifuged at 3100 rpm for 30 minutes at 4°C. 1 ml of supernatant from each well was then transferred to a new 2.4ml Elkay plate, and 1 ml of 70% ethanol was added to each well. The plate was then incubated overnight at -20°C. After overnight incubation the plate was centrifuge cooled to 4°C – 3100 rpm, for 10 minutes. The supernatant was then tipped off and 1.5 ml of ice cold 70% ethanol added to each well. A final round of centrifugation at 3100 rpm, for 10 minutes at 4°C was carried out, and the supernatant tipped off again. The plate was then left to dry at room temperature for 2 hours, then 50 uL of 1x TE buffer was added to each well in order to re-suspend the DNA. Finally, the plate was left at room temperature overnight. Extracted DNA was then quantified using the Nanodrop spectrophotometer and normalised to 5ng/µl. The average ratio of absorbance at 260:280 nm was 1.7 for n=835 samples.

TaqMan genotyping.

Pre-developed assays were used to type 34/37 SNP; custom assays were designed for the remaining 3 SNP. Genotyping was carried out using Applied Biosystem's TaqMan SNP genotyping assays: 10ng of DNA was used as template for 2 µl assays (Applied Biosystems, Foster City, CA, USA) performed on the ABI 7900HT platform in 384-well format and analysed with Autocaller software. Typing for two SNP failed (rs6127118, *CYP24A1* and rs11574010, *VDR*); the call rate for the remaining 35 SNP were >95%. Successfully genotyped SNP were tested for deviation from Hardy-Weinberg equilibrium (HWE), stratified by ethnicity, the results of which are presented in Table 2.3. After correction for multiple comparison testing using the Benjamini & Hochberg method with a 5% false discovery rate (230), all alleles conformed to HWE.

Table 2.3. Pearson's Chi-squared test results for Hardy-Weinberg equilibrium in n=835 genotyped SNP.

Gene	SNP [1, 2]	White (n=701)		Asian/British Asian (n=40)		Black /Black British (n=73)		Mixed (n=17)	
		χ^2	P value	χ^2	P value	χ^2	P value	χ^2	P value
CUBN	rs3740165	0.30	0.58	0.07	0.79	0.17	0.68	0.16	0.69
CYP24A1	rs2762939	0.34	0.56	0.35	0.55	0.13	0.72	0.13	0.72
	rs2248137	0.20	0.65	2.44	0.12	0.19	0.66	0.02	0.90
	rs2762934	0.53	0.47	0.01	0.91	1.47	0.23	0.62	0.43
	rs6013897	1.14	0.29	0.14	0.71	0.09	0.77	0.78	0.38
CYP27A1	rs17470271	0.00	0.97	0.03	0.87	0.09	0.76	1.61	0.21
CYP27B1	rs4646537	0.91	0.34	0.01	0.93	0.24	0.63	0.30	0.58
	rs4646536	0.07	0.79	0.01	0.92	0.19	0.66	4.50	0.03
CYP2R1	rs10500804	0.02	0.89	3.11	0.08	0.25	0.62	0.52	0.47
	rs2060793	0.03	0.87	0.30	0.58	0.42	0.52	0.13	0.72
	rs10766197	0.82	0.37	1.62	0.20	0.11	0.74	0.38	0.54
CYP3A4	rs2740574	0.00	0.98	0.18	0.67	0.11	0.74	1.05	0.31
DBP	rs7041	0.63	0.43	3.29	0.07	0.36	0.55	1.89	0.17
	rs4588	0.97	0.32	0.28	0.60	1.11	0.29	0.72	0.40
	rs12512631	0.22	0.64	0.01	0.94	0.25	0.62	0.00	1.00
	rs2070741	1.66	0.20	1.09	0.30	1.27	0.26	6.84	0.01
	rs2298849	0.33	0.57	1.12	0.29	3.08	0.08	0.00	1.00
	rs16846876	3.88	0.05	1.42	0.23	0.36	0.55	1.13	0.29
DHCR7	rs3829251	0.16	0.69	2.81	0.09	0.60	0.44	0.62	0.43
	rs12785878	1.04	0.31	0.53	0.47	3.44	0.06	4.25	0.04
LRP2	rs3755166	0.00	0.98	1.40	0.24	0.00	0.99	0.00	1.00
RXRA	rs7861779	0.04	0.85	0.53	0.47	0.76	0.38	6.38	0.01
	rs9409929	0.84	0.36	0.16	0.69	0.86	0.35	0.00	1.00
VDR	rs4334089	3.93	0.05	0.00	1.00	1.68	0.19	0.28	0.60
	rs10783219	0.13	0.72	2.04	0.15	3.11	0.08	0.57	0.45
	rs4516035	0.52	0.47	0.28	0.59	0.06	0.81	0.78	0.38
	rs11568820	2.82	0.09	1.73	0.19	4.98	0.03	0.00	0.95
	rs7976091	2.32	0.13	1.86	0.17	4.62	0.03	0.22	0.64
	rs2238136	1.15	0.28	0.28	0.60	0.60	0.44	0.72	0.40
	rs1544410	0.44	0.51	0.84	0.36	0.10	0.75	5.16	0.02
	rs2228570	0.39	0.53	4.07	0.04	0.02	0.88	0.00	1.00
	rs2853559	0.18	0.67	0.22	0.64	0.84	0.36	1.47	0.23
	rs7975232	0.03	0.87	0.00	0.96	0.05	0.82	2.21	0.14
	rs7970314	1.58	0.21	0.91	0.34	6.96	0.01	0.46	0.50
	rs731236	0.07	0.80	0.10	0.75	2.69	0.10	0.57	0.45

[1] *After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for interaction remain significant.* ***[2]*** *Ethnicity undefined in n=4.*

Abbreviations: SNP: Single nucleotide polymorphism, $\chi 2$: Chi-squared, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor A, VDR: Vitamin D receptor, CUBN: Cubilin.

2.3.3. Immunological analysis.

Under the supervision of Dr. Claire Greiller (Blizard Institute, Queen Mary University of London) I assisted in the following immunological work.

Preparation of TLR ligands.

TLR ligands (InvivoGen, San Diego, USA) were dissolved according to product inserts to provide stock solutions, before being further diluted using Dulbecco's phosphate buffered saline (D-PBS) (Sigma-Aldrich, St Louis, USA) to produce the required working concentration (Table 2.4). Sterile 96-well polystyrene microplates (Corning Incorporated, Corning, USA) were prepared by adding 20µl of D-PBS or TLR ligand to the appropriate wells, before being stored at -80°C until use.

Table 2.4. Preparation of TLR ligands used to stimulate cytokine release in whole blood samples.

Antigen	Dissolve In	Stock Concentration	Working Concentration	Final Concentration
LPS	1 ml sterile water	5 mg/ml	1 µg/ml	0.1 µg/ml
Pam2CSK4	1 ml endotoxin free water	100 µg/ml	0.1 µg/ml	0.01 µg/ml
Poly I:C	2.5 ml endotoxin free water	10 mg/ml	1 mg/ml	100 µg/ml
R848	500 µl endotoxin free water	1 mg/ml	10 µg/ml	1 µg/ml

Abbreviations: LPS: Lipopolysaccharide, Poly I:C: Polyinosinic:polycytidylic acid, R848: Resiquimod, ml: Millilitre, µg: Microgram, µl: Microlitre.

Multiplex ELISA.

Whole blood collected from study participants was stimulated with TLR ligands and pathogens. 180µl of blood was incubated with 20µl of stimulant or D-PBS in a humidified incubator at 37°C and 5% CO2 for 24 hours. Following this, plasma was aspirated and stored at -80°C until further analysis by multiplex ELISA. A panel of 30 inflammatory mediators were investigated (IL-1β, IL-2, IL-4, IL-5, Il-6, IL-7, IL-8 [CXCL8], IL-10, IL-12, IL-13, IL-15, IL-17, IL-1RA, IL-2R, IFN-α, IFN-γ, TNF-α, MCP-1 [CCL2], MIP-1α [CCL3], MIP-1β [CCL4], RANTES [CCL5], eotaxin [CCL11], MIG [CXCL9], IP-10 [CXCL10], EGF, FGF-

basic, HGF, VEGF, G-CSF, GM-CSF). The Invitrogen Human Cytokine Magnetic 30-plex Panel was used (Invitrogen, Camarillo, CA, USA), following manufacturer's instructions. 25µl of the antibody bead solution was added to each well of a 96-well plate, before washing two times with 200µl wash solution and the addition of 50µl of incubation buffer. Standards were prepared using a serial 1:2 dilution, with 100µl standard or 50µl assay diluents + 50µl sample added to the appropriate wells. Following a 2 hour incubation at room temperature on an orbital shaker, the plate was washed twice with 200µl wash solution, and 100µl of 1x biotinylated detector antibody was added to each well. After a 1 hour incubation at room temperature on an orbital shaker, the plate was washed twice with 200µl wash solution, 100µl of 1x streptavidin-RPE was added to each well, and the plate was incubated for a further 30 minutes. Three washes with 200µl wash solution were carried out, before 125µl wash solution was added to each well to re-suspend the beads. Plates were analysed using the Magpix ® platform (powered by Luminex xmap Technology) and the Luminex xponent ® software.

2.3.4. Gene expression analysis.

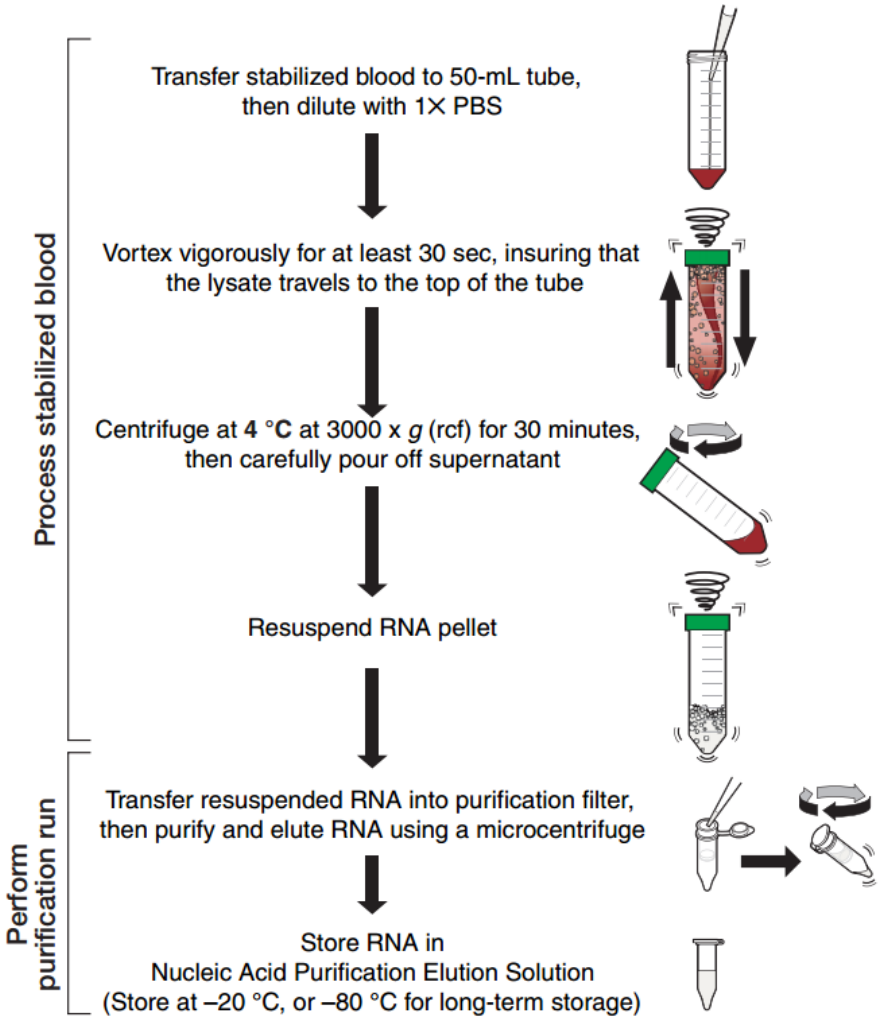
Under the supervision of Dr. Jennifer Roe (Division of Infection & Immunity, University College London) I quantified the relative level of vitamin D receptor gene expression by reverse transcription-quantitative polymerase chain reaction (RT-qPCR), in a subset of n=55 participants from the ViDiCO trial for whom RNA tempus tube blood samples were available, and an association between genotype and the effects of vitamin D supplementation on risk of URI was observed.

RNA extraction.

RNA was extracted from blood tempus tubes following Applied Biosystems' Tempus™ Blood RNA Tube and Tempus™ Spin RNA Isolation Kit protocol (workflow described in Figure 2.3), in batches of 12 samples per run. The following description is for one sample: 3 mL of whole blood was decanted into

a 50 mL tube, into which 6 mL of stabilising solution and 3 mL of PBS was pipetted. The tube was then vortexed for 30 seconds to mix the contents, and centrifuged for 30 minutes (4°C; 3,000 rpm) to separate the RNA. The supernatant was then discarded to leave an RNA pellet and the tube was left inverted on absorbent paper for 2 minutes. 400 µL of RNA purification resuspension solution was then added to the tube after which it was capped and vortexed again to re-suspend the RNA pellet. The tube was then kept on ice whilst performing the following steps: an RNA purification filter was added to a 2 mL Eppendorf tube into which 100 µL of wash solution 1 was pipetted in order to pre-wet the filtration membrane. Roughly 400 uL of the re-suspended RNA was then pipetted into the purification filter and it was then centrifuged for 30 seconds (16,000 x *g*). After this the filter was removed, the waste was discarded and the filter was then re-inserted. 500 µL of wash solution 1 was then pipetted into the purification filter, the tube was centrifuging for 30 seconds (16,000 x *g*), then the purification filter was removed and the waste was discarded. This cycle was repeated a further 2 times, but using wash solution 2, then the empty Eppendorf tube was centrifuged for 30 seconds (16,000 x *g*) to dry the filtration membrane, after which the membrane was transferred to a new 2 mL Eppendorf tube. 100 µL nucleic acid purification elution solution was pipetted into the membrane, and the tube was incubated for 2 minutes at 70°C, followed by another centrifuge for 30 seconds (16,000 x *g*). The filter was then removed and the eluate pipetted back through the membrane again. Following this, the Eppendorf tube was centrifuged one final time for 2 minutes (16,000 x *g*), after which the purification filter was discarded and the remaining ~90 uL of eluate was transferred to a new, labelled Eppendorf tube, being careful to draw the eluate from the surface and not disturb the pellet.

Figure 2.3: The simplified RNA extraction workflow, taken from Applied Biosystem's Tempus™ Blood RNA Tube and Tempus™ Spin RNA Isolation Kit Protocol.



RNA purification.

To remove trace DNA from extracted RNA samples I then used Applied Biosytems' Turbo DNA-free™ kit (revised protocol, 4/2009): Turbo DNase buffer equivalent to 10% RNA sample volume (9 uL) was pipetted into the sample tube and gently mixed, then incubated for 25 minutes at 37°C. DNAse inactivation reagent equivalent to 10% volume of total sample volume (10 uL) was then pipetted into the sample, mixed, then incubated for 5 minutes at room temperature, occasionally mixing

throughout. Finally, the sample was centrifuged for 1.5 minutes and transferred to a new Eppendorf tube.

RNA quantification.

In order to quantify RNA concentration and purity, 1 uL of sample was tested on a Nanodrop spectrophotometer. The average sample concentration was 121.2 ng/uL, and the average absorbance at 260:280 nm wavelength was 1.8, for n=55 samples.

cDNA synthesis.

RNA was reverse transcribed to cDNA using Quanta Biosciences' qScript™ cDNA Synthesis Kit. A cDNA master mix of sufficient volume to run PCR on samples in duplicate for quantification of the target gene and a housekeeping gene (*Glyceraldehyde-3-Phosphate Dehydrogenase [GAPDH]*) was prepared. The per sample reagent volumes were: 5 uL nuclease-free water, 2 uL qScript reaction mix, and 0.5 uL qScript RT (reverse transcriptase and ribonuclease inhibitor protein), for a total volume of 7.5 uL per sample. 7.5 uL of the cDNA master mix was then pipetted into individual 0.2 mL PCR tubes, then 2.5 uL of RNA sample was added and the tubes were then vortexed gently, centrifuged for 10 seconds (16,000 x *g*), and placed in a thermal cycler and ran with the following programme parameters: 1 cycle of 5 minutes at 22°C, 1 cycle of 30 minutes at 42°C, 1 cycle of 5 minutes at 85°C, hold at 4°C.

qPCR.

Comparative qPCR was conducted using Applied Biosystems' TaqMan® Gene Expression Assay for the vitamin D receptor gene (Cat. # 4331182), and *GAPDH* as a housekeeping gene. Each participant sample was run in duplicate for both target and control gene. The experiment was conducted in 96 well plates, which allowed 20 participants to be analysed per run. Two master mixes (one for target gene, one for control gene) were prepared with the following per sample volumes: 5 uL ABI TaqMan MasterMix, 2.4 uL Primer Mix, 0.4 uL Probe, 1.2 uL Nuclease-free water, for a total volume of 9 uL per

well. 9 uL of the appropriate master mix was then pipetted into the appropriate well, to which 1 uL of cDNA sample was added. The plate was then sealed and lightly centrifuged for 10 seconds to mix the reagents, and loaded into an Applied Biosystems 7500 Fast Real-Time PCR System for quantification.

2.4. Statistical analyses.

All analyses were conducted by myself, using STATA (version 12.0, 2011), GraphPad Prism (version 5.03, 2009), and RevMan (version 5.3, 2014). Data were checked for normality by visual inspection of histogram and q-q plots. Normally distributed data were presented as means with a standard deviation (SD), whilst non-normally distributed data were presented as medians with an interquartile range (IQR). Univariate tests were performed for all analyses: Unpaired Student's T tests (2 groups) or one-way ANOVA tests (≥3 groups) were performed on normally distributed dependent variables and Mann-Whitney (2 groups) or Kruskal-Wallis tests (≥3 groups) were performed on non-normally distributed dependent variables in order to identify significantly associated environmental correlates of serum 25(OH)D concentration; clinical correlates of asthma and COPD phenotype; and environmental correlates of ARI and exacerbations of asthma and COPD ($p < 0.05$). All dependent variables were continuous; non-normally distributed dependent variables were transformed to their natural logarithms. All independent variables were classified as categorical variables with median, quartile, or biologically relevant thresholds where converted from continuous data and fitted in multivariable models, providing they had a minimum of 5 participants per subcategory. For genetics analyses, SNP were analysed under an additive model and multiple comparison testing was also applied using the Benjamini & Hochberg method with a false discovery rate (FDR) of 5% (230).

2.4.1. Cross-sectional analyses.

Cross-sectional analyses of environmental and genetic determinants of serum 25(OH)D concentration and the clinical correlates of asthma/COPD phenotype were conducted on all respondents who attended a screening visit for participation in one of our three clinical trials and completed baseline screening questionnaires; underwent anthropometric and clinical measurements; and consented to donate a blood sample for quantification of serum 25(OH)D concentration and for genotyping.

Multivariable linear regression models were used to give adjusted coefficients, along with a 95% confidence interval and P value for pairwise association in variables with 2 categories, or a P value for trend in variables with ≥3 categories. In the case of log-transformed dependent variables, the anti-log of the adjusted regression coefficient is presented. Genetic analyses were adjusted for all significant environmental determinants of serum 25(OH)D concentration that were investigated.

2.4.2. Main effects analyses.

Analyses were performed to investigate the main effects of SNP genotype on risk of ARI and exacerbations of asthma/COPD, independent of vitamin D supplementation. These analyses were by intention to treat in all participants who took at least one dose of study medication and consented to donate a blood sample for quantification of serum 25(OH)D concentration and for genotyping.

Cox regression was performed to give pairwise unadjusted and adjusted hazard ratios, along with a 95% confidence interval for time to first respiratory outcome. These were presented for minor homozygous and heterozygous genotypes, referent to the major homozygous genotype. For adjusted estimates, Cox regression models were corrected for trial stratification and minimisation factors; significant environmental predictors of respiratory outcomes; and study allocation. Adjusted and unadjusted P values for trend were also presented.

2.4.3. Interaction analyses.

Analyses were performed to investigate a possible interaction between: i.) Genotype and study allocation, to see if SNP genotype modifies the effect of vitamin D supplementation on risk of respiratory outcomes. ii.) Between haplotypes and study allocation (where significant SNP in high linkage disequilibrium survive correction for multiple comparisons testing), to see if SNP haplotype

modifies the effect of vitamin D supplementation on risk on respiratory outcomes with a greater level of significance than individual alleles do. iii.) Between significant effect-modifying haplotypes and the concentration of inflammatory mediators released in response to stimulation by toll-like receptor ligands, to see if SNP haplotype modifies the effect of vitamin D supplementation on release of inflammatory mediators.

For analyses i & ii, Cox regression analysis was performed to give hazard ratios along with a 95% confidence interval for the effect of allocation on time to first respiratory outcome within each SNP genotype/haplotype, and a single hazard ratio with a 95% confidence interval and P value for interaction between allocation and SNP genotype/haplotype. These were corrected for trial stratification and minimisation factors and for significant environmental predictors of respiratory outcomes. For analysis iii, multiple linear regression analysis was performed. As the dependent variables (inflammatory mediator concentrations) were non-normally distributed the exponentiated β-coefficient of the interaction term are presented which represents the geometric mean ratio (GMR) of the effect of allocation, for individuals negative or positive for the investigated haplotype. Also presented are P values for interaction between allocation and SNP haplotype i.e. the ratio of geometric mean ratios.

2.4.4. Meta-analysis.

Aggregate data meta-analysis of relevant clinical trials of vitamin D supplementation for prevention of ARI were conducted using a random effects model to account for study heterogeneity. There was no distinction made between upper or lower respiratory tract infections, but selection of trials to enter the meta-analysis was restricted to those which published the proportion of participants who experienced 1 or more ARI events by study arm, and who captured ARI data prospectively and analysed them as their primary outcome. Estimates of the meta-analysis were presented by odds ratio

with a 95% confidence interval for overall effect, with an I^2 percentage for the measure of study heterogeneity.

3. Vitamin D in the prevention of acute respiratory infections: Systematic review of clinical studies.

Before conducting an investigation on the impact genetic variants in the vitamin D pathway impose on vitamin D's ability to prevent ARI, it was prudent to first review the large body of clinical studies which have investigated a direct link between either vitamin D status and risk or ARI, or vitamin D supplementation in the prevention of ARI. This chapter comprises a systematic review and meta-analysis of clinical studies, which I conducted during the first year of my PhD project and was subsequently published in the Journal for Steroid Biochemistry and Molecular Biology in July 2013 (231).

3.1. Introduction.

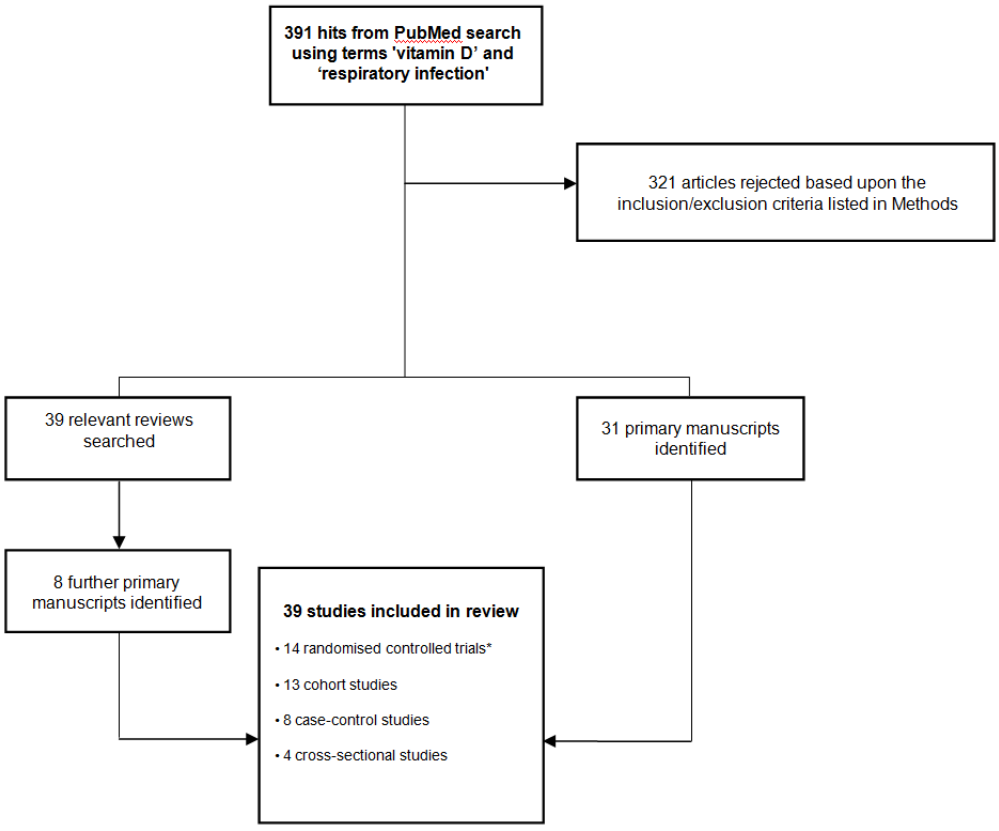
Elucidation of the immunomodulatory actions of vitamin D *in vitro* has prompted the conduct of numerous observational and interventional studies investigating the influence of *in vivo* vitamin D status on susceptibility to a wide range of non-skeletal diseases. Many of these investigations have been distilled by systematic review and meta-analysis, and cover: Cancers (232); Multiple sclerosis (233); Alzheimer's disease (234); Cardiac diseases (235, 236); Diabetes (237); Depression (238); and Tuberculosis (239). Previous *in vitro* work suggests vitamin D may also be an effective intervention for ARI. The 1-alpha hydroxylase enzyme, CYP27B1, that catalyses 25[OH]D to 1,25(OH)2D has been found to be expressed locally in extra-renal tissues, including leucocytes and pulmonary epithelium, and its expression is induced by both viral and bacterial ligands (113, 116). Thus, $1,25(OH)_2D$ can be synthesised in the lung in response to pulmonary infection when 25(OH)D substrate is available (i.e. in vitamin D replete individuals). VDR is widely expressed in cells of the immune system and the respiratory tract, and $1,25(OH)_2D$ ligates it to induce broad-spectrum antimicrobial responses that are effective against both viral and bacterial respiratory pathogens (240, 241). At the time of writing this review, in early 2012, a wealth of observational studies investigating an association between vitamin D deficiency and risk of ARI had been conducted, and so had a respectable number of clinical studies which administered vitamin D dosing regimens of ranging sizes and frequency for the prevention of ARI. A synthesis of these studies had not been previously undertaken however, therefore this chapter presents the first systematic review and meta-analysis of clinical studies, conducted up until September of 2012.

3.2. Results.

3.2.1. Identification and Selection of Studies.

Figure 1 depicts the study selection process. Our initial search identified 391 publications, of which 31 were initially identified as fulfilling eligibility criteria listed in methods, section 2.1.2. A further 8 eligible primary manuscripts were identified from the 39 relevant reviews identified in the initial search, bringing the total number of eligible studies for inclusion in this systematic review to 39.

Figure 3.1. A flow diagram describing the search and study selection process.



3.2.2. Study Characteristics.

Of the 39 studies reviewed, 25 were observational studies (4 cross-sectional, 8 case-control and 13 cohort) and 14 were intervention studies (all randomised controlled trials). The selected studies report data from a total of 47,360 participants whose age ranged from newly born to >80 years old. Studies were conducted in USA (7 studies), UK (4), Canada (3), Japan (3), Afghanistan (2), Finland (2), India (2), New Zealand (2), Bangladesh, Belgium, Ethiopia, Germany, Jordan, Mongolia, the Netherlands, Norway, Poland, Puerto Rico, Romania, Saudi Arabia, Spain and Turkey (1 study each). Thirty-one studies reported serum 25(OH)D concentrations, one study reported serum $1,25(OH)_2D$ concentrations, and seven studies did not report concentrations of either metabolite.

3.2.3. Study Findings.

Table 3.1 presents results of the four cross-sectional studies reviewed: all report consistent associations between low serum 25(OH)D concentrations and increased risk of ARI (242-245).

Results of the eight case-control studies reviewed are presented in Table 3.2: five of these studies report associations between susceptibility to ARI and vitamin D deficiency, as evidenced by the presence of rickets (246, 247) or by low serum 25(OH)D concentrations (248-250); one reports an association between low vitamin D intake and increased risk of LRI (251), and two report no association between serum 25(OH)D concentration and susceptibility to LRI (252, 253).

Table 3.3 presents results of the thirteen cohort studies reviewed: seven report associations between low serum 25(OH)D concentrations and susceptibility to ARI (254-260), two suggest that serum $1,25(OH)_2D$ concentrations may be protective (as evidenced by higher serum $1,25(OH)_2D$ concentrations (261) or by administration of 1-alpha-hydroxylated vitamin D metabolites (262)), three studies were null (201, 263, 264), and one reported a positive association between high maternal

serum 25(OH)D concentration in late pregnancy and increased risk of LRI in offspring during infancy (265).

Table 3.1. Cross-sectional studies investigating association between vitamin D status and susceptibility to ARI and/or exacerbations of asthma, or COPD.

First author, Year, Setting	Participants	Serum 25(OH)D concentration	Main findings
Ginde, 2009. USA (242)	18,883 survey participants, median age 38 years	Median 29.0 ng/ml 3.6% <10 ng/ml 65.1% 10 – 29 ng/ml 31.3% ≥30 ng/ml	• Serum 25(OH)D concentration was inversely associated with risk of self-reported recent URI symptoms (OR 1.36, 95% CI 1.01 to 1.84, for participants with 25(OH)D < 10 ng/ml vs. those with 25(OH)D ≥ 30 ng/ml). • Inverse associations between serum 25(OH)D concentration and risk of URI were stronger in individuals with asthma (OR 5.67) and COPD (OR 2.26).
Jarrti, 2010, USA (243)	284 hospitalised wheezing children, median age 1.6 years	Mean 27.2 ng/ml	Serum 25(OH)D concentration was inversely associated with risk of RSV infection (OR per 4 ng/ml increase, 0.91; 95% CI 0.83 to 0.99), rhinovirus (OR per 4 ng/ml increase, 0.92; 95% CI 0.85 to 0.99) and multiple viral cause (OR per 4 ng/ml increase, 0.91; 95% CI 0.84 to 0.99).
Berry, 2011. UK (244)	6,789 survey participants aged 45 years	Mean 29.0 ng/ml 7.7% <10 ng/ml 69.8% 10-29 ng/ml 22.6% ≥30 ng/ml	Serum 25(OH)D was inversely associated with risk of acute respiratory infection (after adjustment for adiposity, lifestyle and socio-economic factors, each 4 ng/ml increase in 25(OH)D associated with a 7% lower risk of self-reported ARI; 95% CI, 3% to 11%; P for trend < 0.001).
Brehm, 2012. Puerto Rico (245)	287 children with asthma aged 6-14 years	Mean 32.0 ng/ml 44% < 30 ng/ml 56% ≥ 30 ng/ml	Serum 25(OH)D concentration < 30 ng/ml associated with higher odds of ≥1 severe asthma exacerbation in the prior year in multivariate analysis (OR 2.6, 95% CI 1.5 to 4.9, P=0.001)

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, URI: Upper respiratory tract infection, OR: odds ratio, CI: Confidence interval, RSV: Respiratory syncytial virus, ARI: Acute respiratory infection, COPD: Chronic obstructive pulmonary disease, ng/ml: nanograms per millilitre. 25(OH)D concentrations converted from nanomoles per litre (nmol/L) to ng/ml by dividing by 2.496.

Table 3.2. Case-control studies investigating association between vitamin D status and susceptibility to ARI and/or exacerbations of asthma, or COPD.

First author, Year, Setting	Participants	Mean serum 25(OH)D concentration		Main findings
Muhe, 1997. Ethiopia (246)	1000 children, mean age 13 months: 500 rickets cases vs. 500 healthy controls.	Not measured		Diagnosis of rickets associated with susceptibility to pneumonia (adjusted OR 13·37; 95% CI, 8·08 to 24·22; P<0·001).
Najada, 2004. Jordan (247)	443 acutely hospitalised children aged 3-24 months: 47 cases vs. 396 controls without rickets.	Not measured		Diagnosis of rickets associated with risk of LRI (85% of children with rickets had LRI vs. 30% of children without rickets, P<0.01)
Wayse, 2004. India (248)	150 children, mean age 23.9 months: 80 cases with LRI vs. 70 healthy controls.	Cases, 9.1 ng/ml	Controls, 15.4 ng/ml	• Serum 25(OH)D concentration was significantly lower in cases vs. controls (P<0.001). • Serum 25(OH)D concentration > 9.0 ng/ml associated with decreased risk of LRI (adjusted OR 0.09; 95% CI, 0.03 to 0.24; P<0.001).
Karatekin, 2009. Turkey (249)	40 neonates: 25 cases admitted to neonatal intensive care with LRI vs. 15 healthy controls.	Cases , 9.1 ng/ml	Controls, 16.3 ng/ml	• Mean serum 25(OH)D concentration was significantly lower in cases with LRI vs. healthy controls (P=0.01). • Serum 25(OH)D < 10 ng/ml associated with increased risk of LRI (OR 4.25; 95% CI, 1.06 to 17.07; P=0.04)
Roth, 2009. Canada (252)	129 children, mean age 13 months: 64 cases hospitalised with uncomplicated LRI vs. 65 healthy controls.	Cases, 30.9 ng/ml	Controls, 30.8 ng/ml	• No significant difference in mean serum 25(OH)D concentration between LRI cases vs. controls (P=0.96). • Inadequate vitamin D status was not associated with the risk of LRI at either 16 ng/ml or 32 ng/ml 25(OH)D thresholds (P≥0.37).
McNally, 2009. Canada (253)	197 children, mean age 14 months: 105 cases hospitalised with LRI vs. 92 controls attending hospital with other diagnosis	Cases, 32.5 ng/ml	Controls, 33.3 ng/ml	• No significant difference in mean serum 25(OH)D concentration between cases vs. controls (P=0.71). • Among cases, serum 25(OH)D < 20 ng/ml associated with increased risk of admission to the intensive care unit (adjusted OR 8.23, 95% CI, 1.4 to 48.0, P=0.02)
Roth, 2010. Bangladesh (250)	50 children aged 1-18 months: 25 cases hospitalised with LRI vs. 25 healthy controls.	Cases, 11.7 ng/ml	Controls, 15.7 ng/ml	• Mean serum 25(OH)D concentration was significantly lower in LRI cases vs. healthy controls (p=0.015). • Adjusted odds for LRI was reduced 4.3-fold for every 4 ng/ml increase in serum 25(OH)D concentration (adjusted OR 0.23; 95% CI, 0.06 to 0.81; P=0.02).
Leis, 2012. Canada (251)	197 children aged <5 years: 105 cases hospitalised with LRI vs. 92 controls attending hospital with other diagnosis.	Not presented		Vitamin D intake <80 IU/kg/day associated with increased risk of LRI (adjusted OR 4.9, 95%CI 1.5-16.4, P=0.01).

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, OR: Odds ratio, CI: Confidence interval, LRI: Lower respiratory tract infection, ng/ml: nanograms per millilitre, IU: International units, Kg: kilograms. 25(OH)D concentrations converted from nanomoles per litre (nmol/L) to ng/ml by dividing by 2.496.

Table 3.3. Cohort studies investigating association between vitamin D status and susceptibility to ARI and/or exacerbations of asthma, or COPD.

First author, Year. Setting	Design	Participants	Duration follow-up	Serum 25(OH)D concentration	Main findings
Laaksi, 2007. Finland (254)	Prospective	756 male military recruits, aged 18-29 years.	6 months	Mean, 32.1 ng/ml; 3.6% < 16 ng/ml	Subjects with 25(OH)D <16 ng/ml had more days of absence from duty due to respiratory infection than those with 25(OH)D ≥16 ng/ml (incidence rate ratio 1.63; 95% CI, 1.15 to 2.24, P=0.004).
Gale, 2008. UK. (265)	Prospective (birth cohort)	466 mothers (mean age not reported) and 466 infants.	9 months	Mean maternal 25(OH)D at late pregnancy, 20 ng/ml; 21.2% <11 ng/ml; 28.3% 11-20 ng/ml; 50.4% >20 ng/ml	• Maternal serum 25(OH)D concentration in the top quartile (>30 ng/ml) vs. bottom quartile (<12 ng/ml) associated with increased risk of pneumonia or bronchiolitis in offspring (OR 4.80, 95% CI 1.01-22.72). • 'Overall' maternal serum 25(OH)D concentration did not associate with risk of ARI in offspring (OR not presented).
Asamura, 2010. Japan (261)	Retrospective	32 nursing home residents, mean age 80.9 years.	2 years	Not presented	• Serum $1,25(OH)_2D$ concentration inversely associated with risk of febrile respiratory illness (64% in those with $1,25(OH)_2D$ <42 pg/ml vs. 22% in those with $1,25(OH)_2D$ ≥42 pg/ml, P=0.03). • Serum 1,25(OH)2D concentration did not significantly associate with risk of pneumonia (21% in those with $1,25(OH)_2D$ <42 pg/ml vs. 6% in those with $1,25(OH)_2D$ ≥42 pg/ml, P=0.30).
Sabetta, 2010. USA (255)	Prospective	198 healthy adults aged 20-88 years.	4 months	Mean, 28.4 ng/ml; 90.9% <38 ng/ml; 9.1% ≥38 ng/ml	• Serum 25(OH)D concentration inversely associated with risk of viral ARTI (45% in those with 25(OH)D <38 ng/ml vs. 17% in those with serum 25(OH)D ≥38 ng/ml, P=0.01). • Serum 25(OH)D concentration did not associate with median illness duration (6 days in those with serum 25(OH)D <38 ng/ml vs. 6 days in those with serum 25(OH)D ≥38 ng/ml (P value not presented).
Brehm, 2010. USA (260)	Prospective	1,024 children with mild-moderate asthma, median age 8.9 years	4 years	35% ≤ 30 ng/ml; 65% > 30 ng/ml	Serum 25(OH)D concentration ≤ 30 ng/ml associated with higher odds of any hospitalization or emergency department visit (OR 1.5; 95% CI, 1.1 to 1.9; P=0.01).
Camargo, 2011. New Zealand (256)	Prospective (birth cohort)	922 neonates	5 years	Cord blood concentrations: Median, 17.6 ng/ml; 19.5% <10 ng/ml; 53.3% 10-29 ng/ml; 27.2% ≥30 ng/ml	• Serum 25(OH)D concentration inversely associated with risk of ARI by 3 months of age (OR 1.0 for ≥30 ng/ml, 1.39 for 10-30 ng/ml, 2.16 for 25(OH)D < 10 ng/ml, P for trend 0.004). • Cord-blood 25(OH)D levels inversely associated with risk of wheezing by 15 months, 3 years and 5 years of age (P<0.05)
Tsujimoto, 2011. Japan (262)	Retrospective	508 haemodialysis patients, mean age 59.6 yrs. 212 took alfacalcidol or	5 years	Not presented	Administration of alfacalcidol or calcitriol was associated with reduced risk of hospitalisation with LRI (adjusted hazard ratio: 0.47; 95% CI 0.25 - 0.90; P=0.02).

Table 3.3 continued.

First author, Year. Setting	Design	Participants	Duration follow-up	Serum 25(OH)D concentration	Main findings
		calcitriol, 296 did not.			
Belderbos, 2011. Netherlands (257)	Prospective (birth cohort)	156 neonates	1 year	Mean, 32.9 ng/ml; 23.1% <20 ng/ml; 30.8% 20-29 ng/ml; 46.1% ≥30 ng/ml	Serum 25(OH)D concentration at birth inversely associated with risk of RSV LRI over 1st year of life (adjusted relative risk 6.2, 95% CI 1.6 to 24.9, P=0.01 for neonates with 25(OH)D <20 ng/ml vs. 25(OH)D≥ 30 ng/ml).
Porojnicu 2012. Romania (263)	Prospective	105 healthy hospital employees, mean age 35.3 years.	2 months	35% <12 ng/ml; 45% 12-19 ng/ml; 17% 20-32 ng/ml; 3% ≥32 ng/ml	Serum 25(OH)D concentration did not significantly associate with self-reported cases of ARI (Spearman coefficient for correlation between 25(OH)D concentration and number of infectious episodes, -0.12; P=0.20).
Morales, 2012. Spain (258)	Prospective (birth cohort)	1,724 infants	6 years	Median maternal 25(OH)D at 12 weeks' gestation, 29.5 ng/ml	Maternal serum 25(OH)D concentration at 12 weeks' gestation inversely associated with risk of LRI in offspring at 1 year (OR 0.67, 95% CI 0.50 to 0.90, P=0.02 for highest vs. lowest quartile of maternal 25(OH)D).
Kunisaki, 2012. USA (264)	Prospective	973 COPD patients	1 year	Mean, 25.7 ng/ml; 8.4% < 10 ng/ml; 23.6% 10-19 ng/ml; 33.1% 20-29 ng/ml; 34.9% ≥ 30 ng/ml	Serum 25(OH)D concentration did not associate with time to first acute exacerbation of COPD (hazard ratio for a 10 ng/ml increment in 25(OH)D, 1.04, 95% CI 0.97 to 1.12)
Mohamed, 2012. Saudi Arabia (259)	Prospective	206 infants	2 years	Mean cord blood concentration, 24.1 ng/ml; 12%<12 ng/ml; 18% 12-19 ng/ml; 26% 20-29 ng/ml; 44% ≥30 ng/ml	• Mean cord blood 25(OH)D concentration was lower among infants who developed LRI in the first 2 years of life vs. those who did not (13.6 ng/ml vs. 28.6 ng/ml, P<0.0001) • In multivariate analysis, low cord blood 25(OH)D concentration independently associated with subsequent risk of LRI (OR 1.08; 95% CI 1.05 to 1.10; P<0.001)
Quint, 2012. UK (201)	Prospective	97 COPD patients	12 months	Median (IQR), Summer: 16.5 ng/ml (10.7 to 26.0 ng/ml); Winter: 11.1 ng/ml (7.8 to 17.8 ng/ml)	Serum 25(OH)D concentration did not associate with risk of COPD exacerbation during summer or winter (median serum 25[OH]D concentration for frequent vs. infrequent exacerbators in summer: 17.7 ng/ml vs. 15.8 ng/ml; winter: 10.0 ng/ml vs. 10.9 ng/ml, respectively; P≥0.21).

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, 1,25(OH)2D: 1,25-dihydroxyvitamin D, OR: Odds ratio; CI: Confidence interval, RTI: Respiratory tract infection, LRI: Lower respiratory tract infection, RSV: Respirartory syncytical virus, ARI: Acute respiratory infection, COPD: Chronic obstructive pulmonary disease, IQR: Interquartile range, ng/ml: nanograms per millilitre. 25(OH)D concentrations converted from nmol/L to ng/ml by dividing by 2.496.

Results of the fourteen clinical trials reviewed are presented in Table 3.4: ARI was primary outcome in eleven of these studies, and a secondary outcome in three. Seven trials reported that vitamin D supplementation protected against ARI – six in the study population as a whole (266-271), and one in a sub-group with profound vitamin D deficiency (272). Six trials reported null effects for all respiratory outcomes investigated (273-278), and one reported a null effect of vitamin D supplementation on primary outcome (pneumonia incidence), with a negative effect on one secondary outcome (vitamin D increased incidence of repeat episodes of radiographically confirmed pneumonia) (279).

Table 3.4. Clinical trials investigating effects of vitamin D supplementation on incidence of ARI and/or exacerbations of asthma, or COPD.

First author, Year, Setting	Participants	Duration of follow-up	Dose of vitamin D_3, intervention arm	Mean serum 25(OH)D concentration		Main findings
Aloia, 2007. USA (266)	208 healthy post-menopausal African-American women aged 50–75 years; 104 allocated to intervention, 104 to placebo.	3 years	800 IU/day for 2 yrs, then 2000 IU/day for 1 year	Intervention: Baseline, 19.3 ng/ml; 3 months, 28.4 ng/ml; 27 months; 34.8 ng/ml	Placebo: Baseline, 17.2 ng/ml; Follow-up: "no significant change throughout the study"	Allocation to intervention arm decreased rate of self-reported URI symptoms (8% intervention arm vs. 25% placebo arm, P<0.002).
Avenell, 2007. UK (273)	3,444 adults aged ≥ 70 years; 1740 allocated to receive vitamin D_3 +/- calcium, 1704 receiving placebo +/-calcium.	2 years	800 IU/day, 1000mg calcium/day, 800 IU/day + 1000mg calcium/day, or placebo	Intervention: Baseline, 15.2 ng/ml (subset of n=60 intervention members); 12 months, 24.8 ng/ml (same subset)	Placebo: Not Presented	Allocation to intervention arm did not affect incidence of self-reported infections (OR 0.90; 95% CI, 0.76 to 1.07; P=0.23) or antibiotic use (OR 0.84; 95% CI, 0.64 to 1.09; P=0.18).
Li-Ng, 2009. USA (274)	162 healthy adults, mean age 59 years; 84 allocated to intervention, 78 allocated to placebo.	3 months	2,000 IU/day	Intervention: Baseline, 25.8 ng/ml; 3 months, 35.5 ng/ml	Placebo: Baseline, 25.2 ng/ml; 3 months, 24.4 ng/ml	Allocation to intervention arm did not affect rate of self-reported URI (12% intervention vs. 14% placebo arm, P=0.56), URI duration (mean duration 5.4 days in intervention arm vs. 5.3 days in placebo arm, P=0.86) or URI severity (mean severity score 2.6 in intervention arm vs. 2.8 in placebo arm, P=0.40).
Bischoff-Ferrari, 2010. Germany (267)	173 patients with recent hip fracture, mean age 84 years; 86 allocated to higher dose vitamin D_3, 87 allocated to lower dose vitamin D_3.	1 year	2,000 IU/day vs. 800 IU/day	Intervention: Baseline, 13.1 ng/ml; 6 months, 45.4 ng/ml; 12 months, 44.7 ng/ml	Placebo: Baseline, 12.1 ng/ml; 6 months, 37.7 ng/ml; 12 months, 35.4 ng/ml	• Allocation to higher dose vitamin D_3 decreased the risk of hospital readmission due to infection at any site (1% in higher dose group vs. 11% in lower dose group; adjusted relative rate difference -90, 95% CI -99 to -13). • Allocation to higher dose vitamin D did not influence risk of hospital readmission due to LRI (0% in higher dose group vs. 2% in lower dose group, P=0.16).
Urashima 2010. Japan (268)	334 school children, aged 6-15 years; 167 allocated to intervention, 167 allocated to placebo.	4 months	1,200 IU/day	Not Presented		• Allocation to intervention arm significantly reduced risk of influenza A infection (10.8% intervention arm vs. 18.6% placebo arm; RR: 0.58; 95% CI, 0.34 to 0.99; P = 0.04).
Laaksi, 2010. Finland (275)	164 military conscripts, aged 18-28 years; 80 allocated to intervention, 84 allocated to placebo.	6 months	400 IU/day	Intervention: Baseline, 31.5 ng/ml; 6 months, 28.7 ng/ml	Placebo: Baseline, 29.8 ng/ml; 6 months, 20.6 ng/ml	• Allocation to intervention arm did not affect the mean number of days absent from duty due to ARI (2.2 in intervention arm vs. 3.0 in placebo arm, P=0.10), or the rate of common cold symptoms (56% intervention arm vs. 52% placebo arm). • Proportion of participants 'remaining healthy' was higher in intervention vs. placebo arm (51% vs. 36% respectively, P=0.05).
Manaseki-Holland, 2010. Afghanistan (269)	453 children diagnosed with pneumonia, aged 1-36 months; 224 allocated to intervention, 229 allocated to placebo.	3 months	Single bolus dose of 100,000 IU	Not Presented		• Allocation to intervention arm reduced the risk of repeat LRI episode within 90 days of randomisation (RR 0.78; 95% CI, 0.64 to 0.94; P=0.01). • Allocation to intervention arm did not affect the mean number of days to recovery (4.7 days in intervention arm vs. 5.0 days in placebo arm; P=0.17).

Table 3.4 continued.

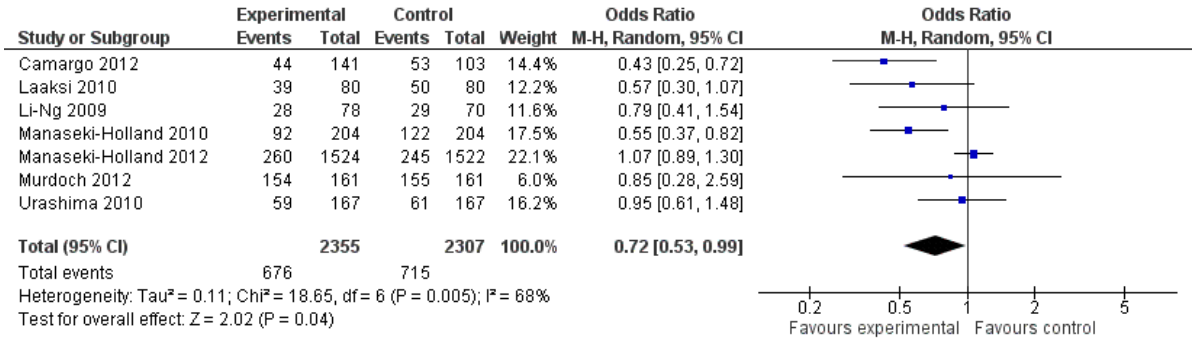
First author, Year, Setting	Participants	Duration of follow-up	Dose of vitamin D_3, intervention arm	Mean serum 25(OH)D concentration		Main findings
Kumar, 2011. India (276)	2,079 low birthweight infants born at >37 weeks' gestation; 1,039 allocated to intervention, 1,040 allocated to placebo.	6 months	1,400 IU/week	Intervention: Baseline, Not Presented; 6 months, 22.0 ng/ml	Placebo: Baseline, Not Presented; 6 months, 14.4 ng/ml	Allocation to intervention arm did not affect incidence of pneumonia or incidence of all-cause hospital admission or death (adjusted rate ratio 0.98, 95% CI 0.70 to 1.38, P=0.92).
Majak, 2011. Poland (270)	48 children with budesonide-treated asthma aged 5-18 years; 24 allocated to intervention, 24 allocated to placebo.	6 months	500 IU/day	Intervention: Baseline, 36.1 ng/ml; 6 months, 37.6 ng/ml	Placebo: Baseline, 35.1 ng/ml; 6 months, 31.9 ng/ml	Allocation to the intervention arm significantly decreased the risk of asthma exacerbation (17% intervention arm vs. 46% placebo arm, P=0.03).
Lehouck, 2012. Belgium (272)	182 patients with moderate to severe COPD, mean age 68 years; 91 allocated to intervention, 91 allocated to placebo.	1 year	Monthly bolus dose of 100,000 IU	Intervention: Baseline, 20.1 ng/ml; 12 months, 52.0 ng/ml	Placebo: Baseline, 19.8 ng/ml; 12 months, 20.4 ng/ml	• Allocation to intervention arm did not influence time to first exacerbation (HR 1.10; 95% CI, 0.82 to 1.56, P=0.41) • Subgroup analysis of participants with baseline 25(OH)D <10 ng/ml (n = 30) showed reduced annual exacerbation rate in the intervention arm (rate ratio 0.57, 95% CI 0.33 to 0.98, P=0.04)
Manaseki-Holland, 2012. Afghanistan (279)	3,046 children diagnosed with pneumonia, aged 1-11 months; 1,524 allocated to intervention, 1522 allocated to placebo.	18 months	3-monthly bolus dose of 100,000 IU	Intervention: Baseline, Not Presented; 1 week, 51.9 ng/ml; 1.5 months, 30.6 ng/ml; 3 months, 22.2 ng/ml; 6.5 months, 42.0 ng/ml; 22 months, 20.8 ng/ml	Placebo: Baseline, Not Presented; 1 week, 17.2 ng/ml; 1.5 months, 13.2 ng/ml; 3 months, 15.9 ng/ml; 6.5 months, 21.2 ng/ml; 22 months, 20.1 ng/ml	Allocation to intervention arm did not affect the incidence of first or only pneumonia (incidence rate ratio 1.06, 95% CI 0.89 to 1.27, P=0.48), but did increase incidence of repeat episodes of radiographically – confirmed pneumonia (incidence rate ratio 1.69, 95% CI 1.28 to 2.21, P<0.0001)
Jorde, 2012. Norway (277)	569 participants of 10 different clinical trials, median age 63 years; 289 allocated to intervention, 280 allocated to placebo.	6 months	1111-6800 IU/day	Not Presented		Allocation to intervention arms did not influence risk of influenza-like illness (13% intervention arms vs. 15% placebo arms, P=0.52)
Camargo, 2012. Mongolia (271)	247 schoolchildren, mean age 10 years; 141 allocated to intervention, 103 to placebo.	7 weeks	300 IU/day	Intervention: (median) Baseline, 7 ng/ml; 7 weeks, 18.9 ng/ml	Placebo: (median) Baseline, 6.8 ng/ml; 7 weeks, 7.2 ng/ml	Allocation to intervention arm vs. placebo arm halved the rate of maternally reported ARI (adjusted RR: 0.50, 95% CI 0.28 to 0.88).
Murdoch, 2012. New Zealand (278)	322 healthy adults, mean age 47 years; 161 allocated to intervention, 161 allocated to placebo.	18 months	Bolus dose of 200,000 IU in months 1 and 2, bolus dose of 100,000 IU/month thereafter.	Intervention: Baseline, 29 ng/ml; 18 months, ~50 ng/ml	Placebo: Baseline, 28 ng/ml; 18 months, ~22 ng/ml	Allocation to intervention arm had no effect on the number of URI (RR: 0.97; 95% CI, 0.85-1.11) or duration of URI symptoms (RR:0.96; 95% CI, 0.81-1.30).

Abbreviations: URI: Upper respiratory tract infection, LRI: Lower respiratory tract infection, ARI: Acute Respiratory Infection, HR: Hazard ratio, RR: Risk ratio, COPD: Chronic obstructive pulmonary disease, IU: International units, CI: confidence interval, ng/ml: nanograms per millilitre. 25(OH)D concentrations converted from nanomoles per litre (nmol/L) to ng/ml by dividing by 2.496.

3.2.4. Aggregate data meta-analysis of clinical trials.

Results from aggregate data meta-analysis are presented in Figure 3.2. This was confined to seven clinical trials which present data on the proportion of participants experiencing one or more ARI events which were prospectively captured as the primary outcome. It therefore excludes studies which only present data on total number of ARI events (276, 277); investigated ARI as a secondary outcome (267), or by post-hoc analysis (266, 273); investigated COPD/asthma exacerbations as their primary outcome (272, 280). The summarised estimate for the seven eligible studies indicate that vitamin D supplementation significantly reduces the risk of ARI (OR 0.72; 95% CI 0.53 to 0.99; P=0.04). Despite only including the most comparable studies, a high degree of study heterogeneity was observed (I^2 = 68%), which justifies the use of a random effect model.

Figure 3.2. A forest plot of clinical trials of vitamin D supplementation for prevention of ARI.



3.3. Discussion.

This systematic review has identified broadly consistent evidence of an association between inadequate vitamin D status and susceptibility to ARI in observational studies conducted in large numbers of participants of all ages in diverse geographical settings and with a wide distribution of serum 25(OH)D concentrations. Evidence from intervention studies is more conflicting, with seven of the trials reviewed reporting protective effects of vitamin D supplementation, six reporting null effects, and one reporting an adverse effect of vitamin D supplementation on risk of pneumonia recurrence. Two interpretations of this 'disconnect' in the evidence from observational vs. intervention studies may be made. Associations seen in observational studies may be attributed to confounding, and inconsistent results from trials may be interpreted as providing insufficient evidence of effectiveness of vitamin D supplementation in preventing ARI. Alternatively, it may be argued that associations reported in observational studies are indeed causal, and that negative results in some trials have arisen as a result of high baseline vitamin D status in trial participants and/or ineffective vitamin D supplementation regimens employed in these studies. A consideration of the strengths and limitations of the studies that we have reviewed therefore follows.

3.3.1. Observational Studies: Strengths and Limitations.

Many of the observational studies reviewed here were of good quality, and potential confounders of the relationship between vitamin D deficiency and susceptibility to ARI such as age, sex, season, socioeconomic position and smoking were controlled for in the majority. Cohort studies were well represented, allowing confirmation that vitamin D deficiency precedes the onset of ARI, and does not arise as a consequence of infection, as seen with other micronutrients (281). Most studies reported participants' serum 25(OH)D concentrations, the gold standard measurement of vitamin D status; however, two studies classified participants as vitamin D deficient on the basis of a clinical diagnosis of rickets (246, 247) (which may arise in children with adequate serum 25(OH)D concentrations

(282)), and one reported serum concentrations of 1,25$(OH)_2$D (261) (which are not generally considered to reflect vitamin D status (283)). Although some studies complemented symptom-based case definitions with physician, radiological, serological and/or molecular diagnosis, many utilised symptom-based definitions, which are more subjective and which cannot inform the question of whether protective effects of vitamin D are pathogen-specific, as suggested by some studies (243, 268). It should also be noted that some of the outcomes classified as ARI for the purposes of this review, such as exacerbations of asthma and COPD, do not always have an infectious aetiology.

3.3.2. Intervention Studies: Strengths and Limitations.

All of the clinical trials reviewed here were randomised, double-blind and placebo-controlled: this 'gold standard' study design effectively eliminates the potential for confounding and observer bias to explain any positive findings. However, some important limitations should be noted. First, some trials investigated effects of vitamin D supplementation on ARI as a secondary outcome (267), or in post hoc analyses (266, 273). Where these analyses were not pre-specified in the protocol, false positive results may have arisen as a result of type 1 error, and false negative results may have arisen as a result of type 2 error or as a result of inadequate ascertainment of ARI. In the post hoc analysis conducted by Aloia *et al* (266), for example, the number of URI reported is significantly lower than would be expected for a study population followed over 3 years.

A second limitation relates to the dosing regimens used in some trials: in some cases, these were inadequate to induce prolonged, clinically significant elevations in serum 25(OH)D concentrations among participants in the intervention arm (275, 276). In others, the duration of administration and follow-up was inadequate to allow prolonged vitamin D repletion among participants in the intervention arm (274). A further potential issue concerns the practice of administration of large intermittent bolus doses of vitamin D. This results in a steep and rapid increase in circulating 25(OH)D levels - to supra-physiological concentrations in some cases – followed by a slow decline (284). Such

peaks and troughs could have potentially deleterious effects on the immune response: concentrations of 25(OH)D >56 ng/ml have been associated with impaired immunity to infection (285), possibly reflecting the fact that vitamin D may suppress adaptive responses to infection as well as boost innate responses (286). Moreover, chronic exposure to falling 25(OH)D concentrations has been postulated to cause an imbalance between the activity of enzymes which synthesise and catabolise $1,25(OH)_2D$ in extra-renal tissues, resulting in reduced concentrations of this active metabolite at sites of disease (287). Either or both of these phenomena could have contributed to the excess of recurrent pneumonia observed in the intervention arm of the second trial conducted by Manaseki-Holland *et al* (279). Administration of lower doses of vitamin D at more frequent intervals induces sustained elevation of 25(OH)D concentrations into the physiological range, and this might have more favourable effects on immune function (288). However, if immune defects associated with vitamin D deficiency are mediated via effects on DNA methylation or histone modification, they may not be rapidly reversible by correction of deficiency.

A third limitation relates to the baseline vitamin D status of trial participants: in some cases the minority of participants were deficient (274, 275, 278), while in others (268, 269) baseline vitamin D status was not measured, precluding identification of potentially important sub-group effects. Recently, Lehouck *et al* reported that protective effects of vitamin D supplementation on COPD exacerbation were restricted to participants with baseline 25(OH)D <10 ng/ml (272), suggesting that effects of vitamin D supplementation may be dependent on baseline vitamin D status, and that the 25(OH)D threshold for protection against ARI may be low. Clinical studies conducted in patients with tuberculosis suggest that the influence of vitamin D status on antimicrobial immunity may be modified by genetic variation in the vitamin D receptor (289) and vitamin D binding protein (290); however, none of the studies reviewed investigated this possibility. Additionally, pathogens were not characterised in many of the trials reviewed here, precluding investigation of the possibility that protective effects of vitamin D supplementation are pathogen-specific.

3.3.3. Meta-analysis of clinical trials.

My meta-analysis of clinical trials presenting comparable, prospectively collected event data indicates vitamin D is an effective intervention for prevention of ARI – offering a 28% reduction in risk to members of the treatment arms (OR 0.72; 95% CI 0.53 to 0.99; P=0.04). However, the extremely high level of study heterogeneity (I^2=68%) suggests that contrasting trial characteristics significantly influence the effect of vitamin D, e.g. size of vitamin D dose; use of intermittent bolus dosing vs. daily dosing; proportion of vitamin D deficiency at baseline; and mean participant age. In order to effectively investigate sub-group effects which potentially influence vitamin D's actions a meta-analysis of individual patient data (IPD) is needed. This is a project I am currently co-ordinating (http://www.nets.nihr.ac.uk/projects/hta/-130325) which combines data from over ten thousand participants from 24 relevant clinical trials, and is sufficiently powered to detect clinically important sub-group effects without the limitations of study level heterogeneity.

3.4. Conclusions.

This systematic review has demonstrated broadly consistent associations between vitamin D deficiency and susceptibility to ARI. By contrast, results of vitamin D supplementation trials did not demonstrate consistent protective effects against ARI, but meta-analysis of 7/14 trials with comparable outcomes did indicate vitamin D offers protection from ARI. It is possible that vitamin D is a biomarker for some unknown and un-controlled-for factor, rather than the causative agent responsible for protection from ARI reported in some clinical trials. However, one would expect this unknown factor to be equally distributed across treatment and placebo arms. Or, it may be that studies which report null effects have arisen as a result of sub-optimal vitamin D supplementation regimens and low prevalence of baseline vitamin D deficiency among participants. Thus, further

clinical trials of vitamin D supplementation for the prevention of ARI should be conducted in populations with a high prevalence of deficiency at baseline, using doses sufficient to induce sustained elevation of serum 25(OH)D concentrations, and powered to detect clinically important sub-group effects.

4. Determinants and clinical correlates of vitamin D status in adults with asthma.

This chapter reports the results of a cross-sectional analysis conducted on all individuals who attended a screening visit for participation in the ViDiAs clinical trial (described in Methods, section 2.2.1). The analysis was conducted with a view to identify both the environmental and genetic determinants of vitamin D status in this population, and to test for associations between vitamin D status and clinical markers of asthma severity and control.

4.1. Introduction.

Vitamin D deficiency has been reported to be common among children with asthma in diverse settings, and to associate with reduced forced expiratory volume in one second (FEV_1), poor asthma control and increased requirement for inhaled corticosteroids (ICS) (245, 291-294). Despite the high prevalence of both asthma and vitamin D deficiency among adults in the industrialised world, cross-sectional studies assessing the prevalence, determinants and clinical correlates of vitamin D deficiency in adults with asthma have not previously been performed in such settings to my knowledge. Moreover, despite evidence suggesting that genetic variation can influence vitamin D status in the general population (122), studies to quantify the relationship between single nucleotide polymorphisms (SNP) in the vitamin D pathway and serum 25-hydroxyvitamin D (25[OH]D) concentrations (the accepted biomarker of vitamin D status) have not previously been performed in patients with asthma. Genetic variation in the vitamin D pathway has previously been reported to modify the influence of vitamin D status on the risk of a diverse range of conditions in which deficiency is thought to play a causal role (184, 295) . However, existing studies investigating the potential influence of polymorphisms in the vitamin D pathway on asthma phenotype are few in number (296, 297) and they have not tested for such gene-environment interactions.

I therefore conducted a cross-sectional study to characterise environmental and genetic determinants of vitamin D status in a group of adults with ICS-treated asthma in London, UK; to determine whether serum 25(OH)D concentration or single nucleotide polymorphisms in the vitamin D pathway associate with asthma phenotype as main effects; and to explore whether genetic variation in the vitamin D pathway may modify the influence of serum 25(OH)D concentrations on asthma phenotype.

4.2. Results.

4.2.1. Study population.

A total of 297 adults with a medical record diagnosis of asthma treated with ICS were enrolled in the study between 27th August 2009 and 25th June 2012. All consented to undergo clinical measurements and to donate blood samples for quantification of serum 25(OH)D and PTH concentration; all but one agreed to donate a blood sample for DNA storage and genotyping. Participant characteristics are presented in Table 4.1. Age range was 16-78 years, with a mean of 48.7 years (SD 14.4). Most participants (57.2%) were female. The majority of participants (82.5%) classified their ethnic origin as being White; 8.5% were Black/Black British, 5.7% were Asian/Asian British, and 3.0% were of mixed ethnicity. 261/297 (87.9%) participants' asthma was managed exclusively in primary care. Mean serum 25(OH)D concentration for all participants was 50.6 nmol/L (SD 24.9). Forty participants (13.5%) had serum 25(OH)D concentration <25 nmol/L; 122 (41.1%) had serum 25(OH)D concentration 25 - 49.9 nmol/L; 80 (26.9%) had serum 25(OH)D concentration 50 – 74.9 nmol/L; and only 55 (18.5%) had serum 25(OH)D concentration ≥ 75 nmol/L.

Table 4.1: Characteristics of participants with asthma.

Factor	Category	N=297
Sex, n (%)	Female	170 (57.2)
	Male	127 (42.8)
Mean age, yrs (SD)		48.7 (14.4)
Mean BMI, kg/m^2 (SD)		27.6 (5.9)
Ethnicity, n (%) [1]	White	245 (82.5)
	Asian / Asian British	17 (5.7)
	Black / Black British	25 (8.5)
	Mixed	9 (3.0)
Fitzpatrick skin type, n (%) [2]	1	19 (6.4)
	2	57 (19.2)
	3	127 (42.8)
	4	57 (19.2)
	5	25 (8.4)
	6	12 (4.0)
Socio-economic position, n (%) [3]	1	199 (67.0)
	2	29 (9.8)
	3	29 (9.8)
	4	12 (4.0)
	5	15 (5.0)
	Student	6 (2.0)
	Unemployed	7 (2.4)
Time outdoors per day, n (%)	>2hrs	103 (34.7)
	≤2hrs	194 (65.3)
Vitamin D supplement, IU/day, n (%) [4]	Any	48 (16.2)
	None	242 (81.5)
Quarter of blood draw, n (%)	Q1 (January – March)	88 (29.6)
	Q2 (April – June)	78 (26.3)
	Q3 (July – September)	60 (20.2)
	Q4 (October – December)	71 (23.9)
Smoking status, n (%)	Non-current	279 (93.9)
	Current	18 (6.1)
Mean alcohol intake, units/week (SD) [5]		10.2 (11.7)
Tanning bed use in previous year, n (%)	Yes	19 (6.4)
	No	278 (93.6)
Recent sunny holiday, n (%) [6]	Yes	52 (17.8)
	No	240 (82.2)
BTS step of treatment, n (%)	2: Regular preventer therapy	134 (45.1)
	3: Initial add-on therapy	125 (42.1)
	4: Persistent poor control	35 (11.8)
	5: Continuous / frequent use of OCS	3 (1.0)
Managed exclusively in primary care, n (%)		261 (87.9)
Medication use	Mean ICS dose at entry in beclometasone equivalents, µg (SD) [7]	725.3 (631.2)
	Inhaled LABA use, n (%) [8]	155 (52.2)
	Leukotriene antagonist use, n (%)	33 (11.1)
Mean % predicted FEV_1 (SD)		82.4 (20.3)
Mean FeNO, ppb (SD)		36.2 (26.4)
Mean serum corrected calcium (SD)		2.23 (0.08)
Mean serum PTH (SD)		5.7 (3.9)
Serum PTH >6.8 pmol/L, n (%)	Yes	71 (23.9)
	No	226 (76.1)
Serum 25(OH)D, nmol/L (%)	<25	40 (13.5)
	25 – 49.9	122 (41.1)
	50 – 74.9	80 (26.9)
	≥ 75	55 (18.5)
Mean serum 25(OH)D, nmol/L (SD)		50.6 (24.9)

[1] *Ethnicity not reported in n=1. Mixed ethnicity: n=6 White and Black Caribbean, n=1 British Mauritian, n=1 Asian Caribbean, n=1 Irish Sri Lankan.* ***[2]*** *Fitzpatrick skin-type scores: 1 = extremely fair skin; always burn, never tan, 2 = fair skin; always burn, sometimes tan, 3 = pale skin; sometimes burn, always tan, 4 = olive skin; rarely burn, always tan, 5 = moderately pigmented brown skin; never burn, always tan, 6 = markedly pigmented black skin; never burn, always tan.* ***[3]*** *SEP classes: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations, 6 = student, 7 = Never/Long-term (>5yrs) unemployed.* ***[4]*** *Vitamin D supplement consumption not reported in n=7.* ***[5]*** *One alcohol unit = 8g pure alcohol.* ***[6]*** *Recent sunny holiday not reported in n=5.* ***[7]*** *1µg beclometasone assumed equivalent to 1µg budesonide, 0.5 mcg fluticasone dipropionate and 0.75 mcg ciclesonide.* ***[8]*** *Includes combinations of ICS/LABA and LABA.*

Abbreviations: BMI: Body mass index, OCS: Oral corticosteroids, BTS: British thoracic society, PTH: Parathyroid hormone, FEV_1: Forced expiratory volume in 1 second, LABA: Long acting beta-adrenoceptor agonists, FeNO: Fractional exhaled nitric oxide, ppb: Parts per billion, nmol/L: nanomoles per litre, pmol/L: picomoles per litre, µg: micrograms, SD: Standard deviation.

4.2.2. Environmental determinants of serum 25(OH)D concentration.

Environmental determinants of vitamin D status in participants with asthma are presented in Table 4.2 and Figure 4.1. Multiple linear regression analysis showed the following factors to independently associate with serum 25(OH)D concentration: BMI (adjusted mean difference of 7.2 nmol/L for BMI of ≥25 kg/m^2vs. <25 kg/m^2; 95% CI -13.0 to -1. 5; P=0.014); Ethnicity (adjusted mean difference 13.3 nmol/L for White vs. non-White; 95% CI -25.8 to -0.9; P=0.036); SEP (greatest adjusted mean difference 19.6 nmol/L for SEP groups 1/2 vs. Unemployed; 95% CI -36.8 to -2.5; P for trend = 0.012); Vitamin D supplement consumption (adjusted mean difference 21.5 nmol/L for those taking a dose of any size vs. those taking no vitamin D supplement; 95% CI -28.7 to -14.2; P<0.001); Quarter of blood draw (greatest adjusted mean difference 18.5 nmol/L for those sampled in Q3 vs. Q1; 95% CI -26.2 to -10.8, P for trend <0.001); recent sunny holiday (adjusted mean difference 7.7 nmol/L for those who took one in the previous 2 months vs. those who did not; 95% CI -14.7 to -0.8; P=0.030); and BTS treatment step (adjusted mean difference 8.1 nmol/L for groups 2/3 vs. 4/5; 95% CI -16.0 to -0.3; P=0.043).

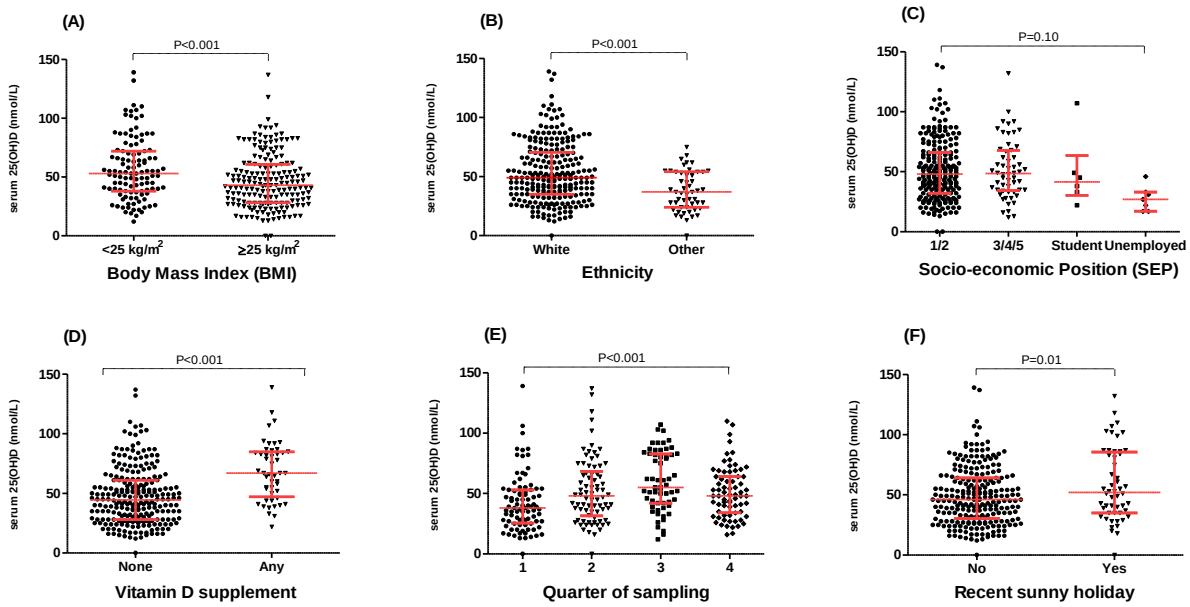
Table 4.2: Environmental determinants of vitamin D status in asthma patients.

Factor	Category	N	Serum 25(OH)D, nmol/L		Univariate P value [7]	Multivariable model - Beta Coefficient (95% CI)	P value [8]
			Mean (SD)	Mean difference			
Sex	Female	170	50.7 (26.1)	referent	0.96	referent	0.13
	Male	127	50.5 (23.2)	-0.2		-4.4 (-10.1 to 1.4)	
Age quartiles	1 (16.0 – 37.7 yrs)	74	52.5 (25.4)	referent	0.26	referent	0.54†
	2 (37.8 – 49.6 yrs)	74	48.4 (25.0)	-4.1		-0.8 (-8.8 to 7.1)	
	3 (49.7 – 60.5 yrs)	74	47.2 (22.2)	-5.3		-0.5 (-8.5 to 7.5)	
	4 (60.6 – 79.0 yrs)	75	54.3 (26.4)	+1.8		+2.6 (-5.6 to 10.8)	
BMI, kg/m²	<25	113	56.8 (26.2)	referent	<0.001	referent	0.014
	≥25	184	46.8 (23.2)	-10.0		-7.2 (-13.0 to -1.5)	
Ethnicity [1]	White	245	53.3 (25.5)	referent	<0.001	referent	0.036
	Non-white	51	37.7 (16.8)	-15.6		-13.3 (-25.8 to -0.9)	
SEP [2]	1,2	228	51.0 (24.9)	referent	0.099	referent	0.012†
	3,4,5	56	51.8 (24.6)	+0.8		+4.9 (-2.0 to 11.8)	
	6 – Student	6	49.0 (30.0)	-2.0		-6.3 (-24.9 to 12.4)	
	7 - Unemployed	7	27.6 (10.3)	-23.4		-19.6 (-36.8 to -2.5)	
Hours spent outdoors/day	>2	103	56.7 (26.9)	referent	0.002	referent	0.17
	≤2	194	47.4 (23.2)	-9.3		-4.1 (-9.8 to 1.7)	
Vitamin D supplement, IU/day [3]	Any	48	68.5 (24.8)	referent	<0.001	referent	<0.001
	None	242	47.4 (23.5)	-21.1		-21.5 (-28.7 to -14.2)	
Quarter of blood draw	Q1 (Jan – Mar)	88	42.2 (23.6)	-17.7	<0.001	-18.5 (-26.2 to -10.8)	<0.001†
	Q2 (Apr – Jun)	78	52.5 (27.1)	-7.4		-11.0 (-19.0 to -3.0)	
	Q3 (Jul – Sep)	60	59.9 (24.2)	referent		referent	
	Q4 (Oct – Dec)	71	51.0 (21.2)	-8.9		-13.5 (-21.6 to -5.4)	
Fitzpatrick skin-type [4]	1,2	76	48.7 (24.4)	-5.3	<0.001	-4.4 (-10.6 to 1.9)	0.81†
	3,4	184	54.0 (25.4)	referent		referent	
	5,6	37	37.6 (17.9)	-16.4		-2.4 (-16.7 to 11.8)	
Smoking status	Non-current	279	50.1 (24.6)	referent	0.22	referent	0.92
	Current	18	57.5 (28.8)	+7.4		-0.6 (-12.3 to 11.0)	
Alcohol consumption, units/wk	0	51	45.0 (23.4)	referent	0.21	referent	0.25†
	1-20	198	51.7 (24.8)	+6.7		+5.7 (-1.9 to 13.2)	
	>20	48	52.0 (26.2)	+7.0		+5.8 (-4.1 to 15.7)	
Tanning bed use, previous year	Yes	19	62.3 (17.9)	referent	0.033	referent	0.19
	No	278	49.8 (25.1)	-12.5		-7.4 (-18.5 to 3.8)	
Recent sunny holiday [5]	Yes	52	58.6 (30.0)	referent	0.012	referent	0.030
	No	240	49.0 (23.5)	-9.6		-7.7 (-14.7 to -0.8)	
BTS treatment step [6]	2/3	259	50.2 (24.0)	-3.1	0.47	-8.1 (-16.0 to -0.3)	0.043
	4/5	38	53.3 (30.4)	referent		referent	

[1] *Ethnicity not reported in n=1. Other ethnicities: n=17 Asian, n=25 Black, n=9 Mixed ethnicity. One participant declined to report ethnicity.* ***[2]*** *Socio-economic Position (SEP) classes: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations, 6 = student, 7 = Never/Long-term (>5yrs) unemployed.* ***[3]*** *Vitamin D supplement consumption not reported in n=7.* ***[4]*** *Fitzpatrick skin-type scores: 1 = extremely fair skin; always burn, never tan, 2 = fair skin; always burn, sometimes tan, 3 = pale skin; sometimes burn, always tan, 4 = olive skin; rarely burn, always tan, 5 = moderately pigmented brown skin; never burn, always tan, 6 = markedly pigmented black skin; never burn, always tan.* ***[5]*** *Recent sunny holiday not reported in n=5.* ***[6]*** *BTS treatment step definitions: 2. Regular preventer therapy, 3. Initial add-on therapy, 4. Persistent poor control, 5. Continuous / frequent use of OCS.* ***[7]*** *Univariate method: Student's T-test/One-way ANOVA.* ***[8]*** *Adjusted for all potential determinants of 25(OH)D concentration included in univariate analysis.* **†** *P-value for trend.*

Abbreviations: CI: Confidence interval, SD: Standard deviation, nmol/L: nanomoles per litre, IU: International units, BTS: British thoracic society.

Figure 4.1. Environmental determinants of vitamin D status in adults with ICS-treated asthma. Lower serum 25-hydroxyvitamin D (25[OH]D) concentrations were associated with higher body mass index (BMI, A), non-white ethnicity (B), lower socio-economic position (C), lack of vitamin D supplement use (D), sampling in Winter or Spring (E) and lack of a recent sunny holiday (F). Error bars represent median and interquartile ranges. P values from linear regression adjusting for all potential environmental determinants of vitamin D status.



4.2.3. Genetic determinants of serum 25(OH)D concentration.

Genetic determinants of vitamin D status are presented in Table 4.3. After adjusting for sex, age, BMI, ethnicity, SEP, hours outdoors, vitamin D supplement consumption, season of blood draw, Fitzpatrick skin-type, smoking status, alcohol consumption, tanning bed use, recent sunny holiday, and BTS treatment step, and correcting for multiple comparison testing, none of our 15 investigated SNP independently predicted serum 25(OH)D concentration.

Table 4.3: Genetic determinants of vitamin D status in asthma patients.

Gene	SNP	Genotype	N	Serum 25(OH)D, nmol/L		Multivariable model - Beta Coefficient (95% CI)	P value for trend [1]
				Mean (SD)	Mean difference		
CYP24A1	rs6013897	TT	174	53.5 (26.6)	referent	Referent	0.13
		AT	96	47.0 (22.5)	-6.5	-3.6 (-9.2 to 2.0)	
		AA	14	41.9 (19.3)	-11.6	-9.4 (-21.4 to 2.6)	
	rs2248137	CC	97	53.4 (25.7)	referent	Referent	0.24
		CG	129	51.2 (26.4)	-2.2	+1.1 (-4.9 to 7.1)	
		GG	60	45.4 (19.6)	-8.0	-4.4 (-11.9 to 3.0)	
DBP	rs16846876	AA	138	54.9 (26.6)	referent	Referent	0.015
		AT	127	47.7 (23.1)	-7.2	-7.8 (-13.2 to -2.4)	
		TT	15	47.5 (19.3)	-7.4	-8.2 (-20.0 to 3.6)	
	rs12512631	TT	113	49.2 (24.9)	referent	Referent	0.087
		CT	137	50.3 (24.2)	+1.1	+2.6 (-3.2 to 8.4)	
		CC	41	56.6 (27.4)	+7.4	+7.2 (-1.0 to 15.4)	
	rs2070741	TT	240	50.2 (24.3)	referent	Referent	0.038
		TG	46	53.7 (25.3)	+3.5	+6.7 (-0.4 to 13.8)	
		GG	1	118.0 (0)	+67.8	+45.7 (2.6 to 88.8)	
	rs2298849	AA	175	50.1 (25.1)	referent	Referent	0.47
		AG	101	51.8 (24.9)	+1.7	+2.9 (-2.8 to 8.5)	
		GG	12	52.2 (24.8)	+2.1	+4.9 (-8.3 to 18.1)	
	rs4588	CC	160	56.0 (25.8)	referent	Referent	0.005
		CA	113	45.0 (23.0)	-11.0	-10.5 (-15.9 to -5.2)	
		AA	16	36.6 (16.6)	-22.4	-16.8 (-28.4 to -5.2)	
	rs7041	GG	91	58.8 (26.7)	referent	Referent	0.053
		TG	132	47.9 (23.1)	-10.9	-8.4 (-14.4 to -2.4)	
		TT	63	47.1 (24.1)	-11.7	-7.6 (-15.2 to 0.1)	
CYP27B1	rs4646536	AA	132	50.9 (25.9)	referent	Referent	0.34
		AG	118	51.8 (25.2)	+0.9	+0.1 (-5.6 to 5.7)	
		GG	30	47.7 (21.4)	-3.2	-4.4 (-13.3 to 4.6)	
CYP2R1	rs10500804	TT	104	54.4 (24.9)	referent	Referent	0.77
		GT	143	46.6 (24.3)	-7.8	-8.1 (-13.8 to -2.4)	
		GG	44	56.5 (25.3)	+2.1	-1.2 (-9.1 to 6.7)	
	rs2060793	GG	107	50.3 (23.9)	referent	Referent	0.84
		AG	129	52.5 (26.3)	+2.2	+2.5 (-3.4 to 8.3)	
		AA	41	45.7 (23.5)	-4.6	-0.8 (-9.0 to 7.4)	
	rs10766197	GG	86	55.0 (24.9)	referent	Referent	0.51
		AG	143	47.4 (24.6)	-7.6	-9.4 (-15.5 to -3.4)	
		AA	43	54.5 (25.4)	-0.5	-2.8 (-11.0 to 5.5)	
DHCR7	rs12785878	TT	154	52.7 (25.3)	referent	Referent	0.011
		GT	98	52.2 (25.1)	-0.5	+1.4 (-4.5 to 7.3)	
		GG	38	38.9 (21.3)	-13.8	-12.2 (-21.5 to -2.8)	
	rs3829251	GG	220	50.1 (23.9)	referent	Referent	0.96
		AG	69	52.6 (27.4)	+2.5	+0.5 (-5.8 to 6.7)	
		AA	2	42.0 (31.1)	-8.1	+0.7 (-30.0 to 31.4)	
VDR	rs10783219	AA	136	50.8 (24.6)	referent	Referent	0.28
		AT	116	50.4 (24.9)	-0.4	-1.8 (-7.6 to 3.9)	
		TT	34	49.3 (28.3)	-1.5	-4.6 (-13.0 to 3.8)	

[1] *Adjusted for sex, age, BMI, ethnicity, SEP, hours outdoors, vitamin D supplement consumption, quarter of sampling, Fitzpatrick skin-type, smoking status, alcohol consumption, tanning bed use, recent sunny holiday, and BTS treatment step. After correction for multiple comparisons testing using the Benjamini & Hochberg method with a 5% false discovery rate, none of the above P values remained significant.*

Abbreviations: DBP: Vitamin D binding protein, CYP-: Cytochrome P450-, DHCR7: 7-dehydrocholesterol reductase, VDR: Vitamin D receptor, SNP: Single nucleotide polymorphism, SD: Standard deviation, CI: Confidence interval, nmol/L: Nanomoles per litre.

4.2.4. Association between vitamin D status and asthma phenotype.

After adjustment for potential confounders, I found no relationship between vitamin D status and asthma phenotype. Specifically, I found no statistically significant independent association between serum 25(OH)D concentration and asthma control test score (Table 4.4), % predicted FEV_1 (Table 4.5), % predicted FVC (Table 4.6), inhaled corticosteroid dose (Table 4.7), FeNO (Table 4.8) or % eosinophils in induced sputum (Table 4.9; n=35 sub-set of participants).

Multiple linear regression analysis did reveal other factors to associate with various aspects of asthma phenotype though. Poor asthma control, as indicated by lower ACT scores, was independently associated with lower alcohol consumption (P for trend = 0.003), BMI ≥25kg/m^2 (P=0.026), non-White ethnicity (P<0.001) and SEP (P for trend = 0.018; Table 4.4). Older age and non-White ethnicity associated with decreased % predicted FEV_1 (P<0.001 for both factors, Table 4.5). Alcohol consumption and previous pneumococcal vaccination associated independently with increased ICS dose (P=0.03 for both factors, Table 4.6). Decreased FeNO levels were associated with female sex (P<0.001) and history of current smoking (P=0.040, Table 4.7), while % eosinophils in induced sputum was positively correlated with % eosinophils in peripheral blood (P=0.013, Table 4.8).

Table 4.4. Environmental determinants of Asthma Control Test (ACT) score.

Factor	Category	N	Median ACT score (IQR)	Univariate P value [1]	Multivariable model – antilog of beta coefficient (95% CI)	P value [2]
Sex	Female	170	19 (16 to 22)	0.031	0.95 (0.89 to 1.00)	0.064
	Male	127	20 (18 to 22)		referent	
Age quartiles	1 (16.0 – 37.7 yrs)	74	20 (18 to 22)	0.96	referent	0.65†
	2 (37.8 – 49.6 yrs)	74	20 (17 to 22)		1.05 (0.97 to 1.14)	
	3 (49.7 – 60.5 yrs)	74	19 (16 to 22)		1.03 (0.95 to 1.11)	
	4 (60.6 – 79.0 yrs)	75	20 (16 to 22)		1.03 (0.94 to 1.12)	
BMI, kg/m^2	<25	113	20 (18 to 22)	0.065	referent	0.026
	≥25	184	20 (16 to 22)		0.94 (0.89 to 0.99)	
Ethnicity [3]	White	245	20 (18 to 22)	<0.001	referent	<0.001
	Other / mixed	51	17 (14 to 21)		0.88 (0.81 to 0.94)	
SEP [4]	1,2	228	20 (18 to 22)	0.011	referent	0.018†
	3,4,5	56	19 (15 to 21)		0.94 (0.87 to 1.00)	
	Student	6	18 (16 to 20)		0.96 (0.79 to 1.16)	
	Unemployed	7	14 (10 to 17)		0.79 (0.67 to 0.94)	
Smoking status	Non-current	279	20 (17 to 22)	0.54	referent	0.27
	Current	18	19 (15 to 22)		0.94 (0.84 to 1.05)	
Alcohol consumption, units/wk [5]	0	44	17 (14 to 21)	<0.001	referent	0.003†
	1-20	198	20 (18 to 22)		1.12 (1.04 to 1.21)	
	>20	48	21 (18 to 23)		1.17 (1.06 to 1.29)	
Influenza vaccination	No	40	20 (16 to 21.5)	0.36	0.97 (0.90 to 1.05)	0.48
	Yes	257	20 (17 to 22)		referent	
Pneumonia vaccination	No	201	20 (17 to 22)	0.71	1.04 (0.98 to 1.10)	0.24
	Yes	96	20 (17 to 22)		referent	
Serum 25(OH)D, nmol/L	<25	40	19.5 (16.5 to 22)	0.87	1.05 (0.96 to 1.16)	0.25†
	25 – 49.9	122	20 (17 to 22)		1.02 (0.94 to 1.09)	
	50 – 74.9	80	20 (17 to 22)		0.99 (0.92 to 1.07)	
	≥ 75	55	20 (17 to 22)		referent	

[1] *Univariate analysis method: Mann-Whitney/Kruskal-Wallis tests.* ***[2]*** *Adjusted for all potential determinants of asthma control test score investigated in univariate analysis.* ***[3]*** *Ethnicity not reported in n=1. Other ethnicities: n=17 Asian / Asian British, n=25 Black / Black British. Mixed ethnicity: n=9.* ***[4]*** *Socio-economic Position (SEP) classes: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations.* ***[5]*** *Alcohol consumption not recorded in n=7.* **†** *P value for trend.*

Abbreviations: BMI: Body mass index, IQR: Interquartile range, CI: Confidence interval, nmol/L: nanomoles per litre.

Table 4.5. Environmental determinants of % predicted Forced Expiratory Volume in 1 second (FEV_1).

Factor	Category	N	Mean % predicted FEV_1 (SD)	Univariate P value [1]	Multivariable model beta coefficient (95% CI)	P value [2]
Sex	Female	170	90.39 (18.37)	0.28	+4.15 (-0.38 to 8.69)	0.073
	Male	127	89.55 (19.80)		Referent	
Age quartiles	1 (16.0 – 37.7 yrs)	74	92.99 (13.64)	0.002	Referent	<0.001†
	2 (37.8 – 49.6 yrs)	74	93.34 (15.26)		+1.55 (-4.55 to 7.65)	
	3 (49.7 – 60.5 yrs)	74	88.04 (22.72)		-5.33 (-11.61 to 0.95)	
	4 (60.6 – 79.0 yrs)	75	83.11 (21.24)		-13.16 (-19.87 to -6.44)	
BMI, kg/m^2	<25	113	91.64 (16.38)	0.10	Referent	0.77
	≥25	184	87.94 (20.36)		-0.69 (-5.23 to 3.85)	
Ethnicity [3]	White	245	92.23 (18.27)	<0.001	Referent	<0.001
	Other / mixed	51	75.36 (16.35)		-17.11 (-23.04 to -11.17)	
SEP [4]	1,2	228	90.71 (18.42)	0.066	Referent	0.36†
	3,4,5	56	84.94 (21.53)		-1.27 (-6.75 to 4.21)	
	Student	6	92.72 (8.84)		-1.09 (-16.00 to 13.83)	
	Unemployed	7	77.33 (15.08)		-6.95 (-20.61 to 6.70)	
Smoking status	Non-current	279	89.24 (19.35)	0.71	Referent	0.35
	Current	18	90.97 (12.71)		-4.22 (-13.13 to 4.69)	
Alcohol consumption, units/wk [5]	0	44	86.93 (20.97)	0.61	Referent	0.98†
	1-20	198	89.96 (18.91)		-0.33 (-6.32 to 5.66)	
	>20	48	90.26 (17.63)		-0.11 (-7.99 to 7.78)	
Influenza vaccination	No	40	89.98 (17.10)	0.82	-0.54 (-6.74 to 5.67)	0.87
	Yes	257	89.25 (19.31)		Referent	
Pneumonia vaccination	No	201	88.83 (18.99)	0.50	-4.09 (-8.99 to 0.81)	0.10
	Yes	96	90.43 (19.08)		Referent	
Serum 25(OH)D, nmol/L	<25	40	85.07 (17.60)	0.48	-1.11 (-8.67 to 6.45)	0.75†
	25 – 49.9	122	89.55 (21.90)		+1.30 (-4.44 to 7.03)	
	50 – 74.9	80	90.33 (15.99)		+1.74 (-4.48 to 7.96)	
	≥ 75	55	90.59 (17.02)		Referent	

[1] *Univariate analysis method: Students T-test / One-way ANOVA tests.* ***[2]*** *Adjusted for all potential determinants of asthma control test score investigated in univariate analysis.* ***[3]*** *Ethnicity not reported in n=1. Other ethnicities: n=17 Asian / Asian British, n=25 Black / Black British. Mixed ethnicity: n=9.* ***[4]*** *Socio-economic Position (SEP) classes: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations.* ***[5]*** *Alcohol consumption not recorded in n=7.* **†** *P value for trend.*

Abbreviations: BMI: Body mass index, SD: Standard deviation, CI: Confidence interval, nmol/L: nanomoles per litre.

Table 4.6. Environmental determinants of % predicted Forced Vital Capacity (FVC).

Factor	Category	N	Mean % predicted FVC (SD)	Univariate P value [1]	Multivariable model – beta coefficient (95% CI)	P value [2]
Sex	Female	170	1.04 (0.17)	0.53	+0.03 (-0.01 to 0.06)	0.14
	Male	127	1.03 (0.15)		Referent	
Age quartiles	1 (16.0 – 37.7 yrs)	74	1.03 (0.12)	0.68	Referent	0.35
	2 (37.8 – 49.6 yrs)	74	1.05 (0.15)		+0.03 (-0.02 to 0.08)	
	3 (49.7 – 60.5 yrs)	74	1.02 (0.18)		-0.01 (-0.06 to 0.04)	
	4 (60.6 – 79.0 yrs)	75	1.03 (0.18)		-0.01 (-0.07 to 0.04)	
BMI, kg/m^2	<25	113	1.07 (0.14)	**0.001**	Referent	**0.004**
	≥25	184	1.01 (0.17)		-0.05 (-0.09 to -0.02)	
Ethnicity [3]	White	245	1.07 (0.15)	**<0.001**	Referent	**<0.001**
	Other / mixed	51	0.88 (0.13)		-0.17 (-0.22 to -0.12)	
SEP [4]	1,2	228	1.05 (0.16)	**0.034**	Referent	0.24
	3,4,5	56	1.00 (0.17)		-0.01 (-0.06 to 0.03)	
	Student	6	0.96 (0.17)		-0.11 (-0.23 to 0.01)	
	Unemployed	7	0.96 (0.13)		-0.04 (-0.15 to 0.07)	
Smoking status	Non-current	279	1.03 (0.16)	0.35	Referent	0.93
	Current	18	1.07 (0.11)		-0.00 (-0.08 to 0.07)	
Alcohol consumption, units/wk [5]	0	44	0.97 (0.17)	**0.012**	Referent	0.16
	1-20	198	1.05 (0.16)		+0.04 (-0.01 to 0.09)	
	>20	48	1.05 (0.14)		+0.05 (-0.06 to 0.11)	
Influenza vaccination	No	40	1.02 (0.16)	0.43	-0.01 (-0.06 to 0.04)	0.63
	Yes	257	1.04 (0.16)		Referent	
Pneumonia vaccination	No	201	1.02 (0.16)	0.068	-0.02 (-0.06 to 0.02)	0.32
	Yes	96	1.06 (0.15)		Referent	
Serum 25-hydroxyvitamin D, nmol/L	<25	40	0.98 (0.13)	0.079	-0.01 (-0.07 to 0.05)	0.81
	25 – 49.9	122	1.04 (0.18)		+0.01 (-0.04 to 0.06)	
	50 – 74.9	80	1.04 (0.15)		+0.00 (-0.05 to 0.05)	
	≥ 75	55	1.07 (0.13)		Referent	

[1] *Univariate analysis method: Students T-test / One-way ANOVA tests.* ***[2]*** *Adjusted for all potential determinants of asthma control test score investigated in univariate analysis.* ***[3]*** *Ethnicity not reported in n=1. Other ethnicities: n=17 Asian / Asian British, n=25 Black / Black British. Mixed ethnicity: n=9.* ***[4]*** *Socio-economic Position (SEP) classes: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations.* ***[5]*** *Alcohol consumption not recorded in n=7. One unit defined as 8g pure alcohol.* ***†*** *P value for trend.*

Abbreviations: BMI: Body mass index, SD: Standard deviation, CI: Confidence interval, nmol/L: nanomoles per litre.

Table 4.7. Environmental determinants of inhaled corticosteroid (ICS) dose.

Factor	Category	N [6]	Median ICS dose (IQR)	Univariate P value [1]	Multivariable model – antilog of beta coefficient (95% CI)	P value [2]
Sex	Female	167	400 (200 to 800)	0.75	1.04 (0.86 to 1.25)	0.71
	Male	127	400 (200 to 800)		referent	
Age quartiles	1 (16.0 – 37.7 yrs)	74	400 (200 to 800)	0.76	referent	0.82†
	2 (37.8 – 49.6 yrs)	74	400 (200 to 800)		0.98 (0.77 to 1.26)	
	3 (49.7 – 60.5 yrs)	72	400 (200 to 800)		0.96 (0.75 to 1.25)	
	4 (60.6 – 79.0 yrs)	74	400 (213.3 to 800)		0.97 (0.74 to 1.28)	
BMI, kg/m^2	<25	113	400 (200 to 600)	0.07	referent	0.29
	≥25	181	400 (250 to 800)		1.11 (0.92 to 1.33)	
Ethnicity [3]	White	243	400 (200 to 800)	0.26	referent	0.21
	Mixed / other	50	400 (300 to 1000)		1.17 (0.92 to 1.49)	
SEP [4]	1,2	225	400 (200 to 800)	0.08	referent	0.081†
	3,4,5	56	400 (400 to 700)		1.13 (0.90 to 1.42)	
	Student	6	300 (200 to 800)		0.88 (0.48 to 1.62)	
	Unemployed	7	800 (400 to 1600)		1.85 (1.06 to 3.24)	
Smoking status	Non-current	276	400 (200 to 800)	0.13	referent	0.028
	Current	18	550 (400 to 800)		1.51 (1.05 to 2.17)	
Alcohol consumption, units/wk [5]	0	43	400 (300 to 800)	0.35	referent	0.95†
	1-20	197	400 (200 to 800)		0.91 (0.71 to 1.16)	
	>20	48	500 (300 to 800)		1.01 (0.73 to 1.40)	
Influenza vaccination	No	40	400 (200 to 500)	0.15	0.89 (0.69 to 1.15)	0.39
	Yes	254	400 (200 to 800)		referent	
Pneumonia vaccination	No	199	400 (200 to 600)	0.01	0.80 (0.65 to 0.97)	0.03
	Yes	95	500 (300 to 800)		referent	
Serum 25(OH)D, nmol/L	<25	40	400 (400 to 500)	0.87	0.98 (0.72 to 1.34)	0.93†
	25 – 49.9	120	400 (200 to 800)		1.00 (0.79 to 1.27)	
	50 – 74.9	80	400 (200 to 700)		1.00 (0.77 to 1.29)	
	≥ 75	54	400 (200 to 800)		referent	

[1] *Univariate analysis method: Mann-Whitney/Kruskal-Wallis tests.* ***[2]*** *Adjusted for all potential determinants of ICS dose investigated in univariate analysis.* ***[3]*** *Ethnicity not reported in n=1. Other ethnicities: n=17 Asian / Asian British, n=25 Black / Black British. Mixed ethnicity: n=9.* ***[4]*** *Socio-economic Position (SEP) classes: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations.* ***[5]*** *Alcohol consumption not recorded in n=7.* ***[6]*** *ICS dose not recorded in n=3.* **†** *P value for trend.*

Abbreviations: BMI: Body mass index, IQR: Interquartile range, CI: Confidence interval, nmol/L: nanomoles per litre.

Table 4.8. Environmental determinants of fractional exhaled nitric oxide (FeNO).

Factor	Category	N [6]	Median FeNO, ppb (IQR)	Univariate P value [1]	Multivariable model – antilog of beta coefficient (95% CI)	P value [2]
Sex	Female	168	25 (17 to 37.5)	<0.001	0.71 (0.61 to 0.83)	<0.001
	Male	126	35.5 (23 to 45)		referent	
Age quartiles	1 (16.0 – 37.7 yrs)	74	31 (20 to 47)	0.49	referent	0.72†
	2 (37.8 – 49.6 yrs)	73	29 (20 to 47)		1.09 (0.88 to 1.34)	
	3 (49.7 – 60.5 yrs)	74	29 (19 to 41)		1.05 (0.84 to 1.30)	
	4 (60.6 – 79.0 yrs)	73	28 (19 to 40)		0.97 (0.77 to 1.22)	
BMI, kg/m^2	<25	113	30 (20 to 47)	0.16	referent	0.16
	≥25	181	29 (19 to 41)		0.89 (0.77 to 1.05)	
Ethnicity [3]	White	243	29 (19 to 42)	0.43	referent	0.96
	Non-white	50	32 (20 to 46)		1.00 (0.81 to 1.22)	
SEP [4]	1,2	226	30 (20 to 42)	0.41	referent	0.19†
	3,4,5	55	25 (17 to 42)		0.84 (0.69 to 1.01)	
	Student	6	29 (14 to 96)		1.09 (0.65 to 1.82)	
	Unemployed	7	39 (23 to 64)		1.28 (0.80 to 2.05)	
Smoking status	Non-current	276	30 (20 to 42.5)	0.22	referent	0.040
	Current	18	24 (13 to 37)		0.72 (0.53 to 0.98)	
Alcohol consumption, units/wk [5]	0	44	27 (18.5 to 45)	0.58	referent	0.21†
	1-20	195	30 (20 to 43)		1.03 (0.84 to 1.27)	
	>20	48	28 (20 to 40)		0.84 (0.64 to 1.10)	
Influenza vaccination	No	40	29.5 (21 to 43)	0.48	1.03 (0.84 to 1.28)	0.75
	Yes	254	29.5 (19 to 42)		referent	
Pneumonia vaccination	No	200	30 (20 to 43.5)	0.34	1.02 (0.86 to 1.21)	0.80
	Yes	94	29 (18 to 41)		referent	
Serum 25(OH)D, nmol/L	<25	40	30 (20.5 to 42)	0.55	1.04 (0.80 to 1.35)	0.84†
	25 – 49.9	120	29 (20 to 44.5)		1.10 (0.90 to 1.34)	
	50 – 74.9	80	31 (19 to 45)		1.13 (0.91 to 1.40)	
	≥ 75	54	24.5 (18 to 37)		referent	

[1] *Univariate analysis method:Mann-Whitney/Kruskal-Wallis tests.* ***[2]*** *Adjusted for all potential determinants of FeNO investigated in univariate analysis.* ***[3]*** *Ethnicity not reported in n=1. Other ethnicities: n=17 Asian / Asian British, n=25 Black / Black British. Mixed ethnicity: n=9.* ***[4]*** *Socio-economic Position (SEP) classes: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations.* ***[5]*** *Alcohol consumption not recorded in n=7.* ***[6]*** *Exhaled nitric oxide levels not recorded in n=3.* **†** *P value for trend.*

Abbreviations: BMI: Body mass index, IQR: Interquartile range, CI: Confidence interval, nmol/L: nanomoles per litre.

Table 4.9. Environmental determinants of % eosinophils in sputum (subset of n=35 participants).

Factor	Category	N	Median % eosinophils (IQR)	Univariate P value[1]	Multivariate model – antilog of beta coefficient (95% CI)	P value[2]
Sex	Female	17	1.54 (0.75 to 3.00)	0.62	0.82 (0.50 to 1.34)	0.40
	Male	18	1.50 (0.63 to 4.42)		referent	
Age quartiles	1 (16.0 – 37.7 yrs)	7	1.00 (0.58 to 1.50)	0.49	Referent	0.67†
	2 (37.8 – 49.6 yrs)	6	3.09 (1.25 to 5.50)		1.53 (0.70 to 3.38)	
	3 (49.7 – 60.5 yrs)	10	1.50 (0.63 to 2.75)		1.03 (0.49 to 2.14)	
	4 (60.6 – 79.0 yrs)	12	1.71 (0.63 to 4.25)		0.96 (0.43 to 2.13)	
BMI, kg/m^2	<25	11	1.50 (0.42 to 2.00)	0.21	referent	0.13
	≥25	24	1.54 (0.71 to 5.25)		1.54 (0.87 to 2.70)	
Blood eosinophilia	<3%	17	0.83 (0.58 to 1.50)	0.006	referent	0.013
	≥3%	18	2.88 (1.50 to 6.50)		2.02 (1.18 to 3.47)	
FEV_1/FVC	<0.93	19	1.75 (1.17 to 5.00)	0.11	referent	0.32
	≥0.93	16	0.92 (0.50 to 1.92)		0.76 (0.44 to 1.33)	
Serum 25(OH)D, nmol/L	<50	19	1.50 (0.63 to 4.42)	0.97	1.03 (0.58 to 1.84)	0.91
	≥ 50	16	1.50 (0.63 to 3.25)		referent	

[1]*Univariate analysis method:Mann-Whitney/Kruskal-Wallis tests.* ***[2]*** *Adjusted for all potential determinants of sputum eosinophilia investigated in univariate analysis.* **†** *P value for trend.*

Abbreviations: BMI: Body mass index, IQR: Interquartile range, CI: Confidence interval, nmol/L: nanomoles per litre, FEV1/FVC: Forced expiratory volume in 1 second to forced vital capacity ratio.

4.2.5. Association between genetic factors and asthma phenotype.

Finally, I investigated whether 35 SNP that had been previously been reported to associate with serum 25(OH)D concentrations and/or risk of non-skeletal diseases associated with % predicted FEV1 (Table 4.10), % predicted FVC (Table 4.11), FeNO (Table 4.12), ACT score (Table 4.13), or ICS requirement (Table 4.14). After correcting for multiple comparisons testing (Benjamini & Hochberg method with a 5% false discovery rate) none of the SNP investigated were found to associate with any outcome, either as main effects, or by interaction with baseline vitamin D status.

Table 4.10. Genetic determinants of Forced Expiratory Volume in 1 second (FEV_1).

Gene	SNP	Genotype	N	Mean FEV_1 (SD)	Multivariable model: beta coefficient (95% CI) [1]	P value for trend	P value for genotype* 25(OH)D interaction
CYP3A4	rs2740574	AA	249	90.1 (19.4)	referent	0.30	0.25
		AG	33	86.6 (16.2)	+3.65 (-3.80 to 11.10)		
		GG	10	76.1 (15.6)	+6.96 (-6.18 to 20.09)		
CUBILIN	rs3740165	TT	259	89.1 (19.0)	referent	-	-
		TC	29**	90.3 (20.5)	-		
		CC	0*	-	-		
RXRA	rs7861779	GG	182	90.0 (19.2)	referent	0.42	0.20
		GA	86	88.4 (19.5)	+1.56 (-3.26 to 6.39)		
		AA	17	82.8 (17.2)	+4.01 (-5.80 to 13.81)		
	rs9409929	GG	125	89.3 (18.3)	referent	0.68	0.89
		AG	127	89.5 (19.7)	+0.39 (-4.14 to 4.91)		
		AA	35	90.1 (18.4)	-1.43 (-8.28 to 5.42)		
CYP24A1	rs6013897	TT	174	90.2 (19.0)	referent	0.11	0.85
		AT	96	88.7 (18.8)	-1.94 (-6.40 to 2.53)		
		AA	14	82.0 (21.7)	-8.03 (-17.83 to 1.77)		
	rs2762934	GG	206	90.1 (19.6)	referent	0.93	0.37
		AG	77	87.3 (17.4)	-2.27 (-7.07 to 2.53)		
		AA	7	88.5 (25.1)	-0.57 (-14.18 to 13.03)		
	rs2762939	GG	159	91.0 (19.3)	referent	0.70	0.043
		CG	105	86.6 (17.9)	-2.42 (-6.93 to 2.08)		
		CC	22	88.0 (21.7)	-1.57 (-9.68 to 6.53)		
	rs2248137	CC	97	90.2 (19.3)	referent	0.90	0.28
		CG	129	89.9 (18.6)	-0.56 (-5.37 to 4.26)		
		GG	60	86.0 (19.3)	+0.38 (-5.65 to 6.41)		
DBP	rs16846876	AA	138	87.4 (19.4)	referent	0.019	0.47
		AT	127	89.7 (18.1)	-0.14 (-4.56 – 4.28)		
		TT	15	98.6 (15.7)	+11.34 (1.85 to 20.83)		
	rs7041	CC	91	93.3 (18.6)	referent	0.32	0.86
		AC	132	88.4 (18.8)	-2.94 (-7.95 to 2.08)		
		AA	63	84.9 (19.9)	-3.15 (-9.32 to 3.02)		
	rs12512631	TT	113	89.6 (18.4)	referent	0.92	0.78
		CT	137	88.0 (20.6)	-1.43 (-5.98 to 3.11)		
		CC	41	91.8 (15.5)	+0.32 (-6.16 to 6.79)		
	rs4588	GG	160	89.3 (18.3)	referent	0.20	0.76
		GT	113	88.1 (20.2)	-2.91 (-7.50 to 1.68)		
		TT	16	96.1 (19.8)	+6.22 (-3.29 to 15.73)		
	rs2070741	TT	240	89.3 (19.6)	referent	0.038	0.14
		TG	46	89.0 (15.7)	+1.34 (-4.45 to 7.12)		
		GG	1*	-	-		
	rs2298849	AA	175	87.8 (18.8)	referent	0.12	0.99
		AG	101	91.5 (19.6)	+3.86 (-0.63 to 8.35)		
		GG	12	93.4 (15.3)	+8.37 (-2.21 to 18.96)		
CYP27A1	rs17470271	AA	113	88.3 (18.9)	referent	0.97	0.46
		AT	133	89.1 (19.7)	-1.46 (-6.15 to 3.23)		
		TT	44	91.8 (17.6)	+0.14 (-6.33 to 6.61)		
CYP27B1	rs4646536	AA	132	89.5 (18.9)	referent	0.11	0.42
		AG	118	89.0 (19.3)	+0.60 (-3.90 to 5.10)		
		GG	30	86.9 (19.9)	-5.86 (-13.01 to 1.29)		
	rs4646537	TT	264	89.4 (18.8)	referent	0.44	0.68
		GT	26	87.6 (22.2)	+2.97 (-4.58 to 10.51)		
		GG	0*	-	-		
CYP2R1	rs10500804	TT	104	88.6 (18.9)	referent	0.64	0.90
		GT	143	89.4 (19.4)	-0.53 (-5.25 to 4.19)		
		GG	44	90.0 (18.7)	-1.57 (-8.11 to 4.97)		
	rs2060793	GG	107	86.8 (19.2)	referent	0.32	0.55

Table 4.10 continued.

Gene	SNP	Genotype	N	Mean FEV_1 (SD)	Multivariable model: beta coefficient (95% CI) [1]	P value for trend	P value for genotype* 25(OH)D interaction
		AG	129	90.3 (19.4)	+2.69 (-2.00 to 7.37)		
		AA	41	89.9 (18.0)	+3.33 (-3.21 to 9.87)		
	rs10766197	GG	86	88.0 (19.7)	referent	0.59	0.50
		AG	143	89.2 (19.1)	-0.02 (-5.07 to 5.02)		
		AA	43	89.6 (18.9)	-1.85 (-8.65 to 4.95)		
LRP2	rs3755166	GG	101	87.7 (18.4)	referent	0.82	0.99
		AG	151	90.2 (20.1)	+0.45 (-4.31 to 5.21)		
		AA	38	90.6 (16.5)	-0.81 (-7.72 to 6.10)		
DHCR7	rs12785878	TT	154	93.1 (18.3)	referent	0.72	0.37
		GT	98	86.6 (19.9)	-3.06 (-7.87 to 1.75)		
		GG	38	81.7 (16.5)	-1.36 (-8.91 to 6.20)		
	rs3829251	GG	220	89.3 (19.1)	referent	0.41	0.067
		AG	69	89.3 (19.4)	+3.13 (-1.96 to 8.22)		
		AA	2	96.3 (12.0)	+10.47 (-14.58 to 35.51)		
VDR	rs731236	AA	113	87.8 (17.8)	referent	0.60	0.37
		AG	132	90.9 (20.2)	+1.78 (-2.79 to 6.36)		
		GG	42	88.5 (19.2)	+1.76 (-4.76 to 8.27)		
	rs4334089	GG	145	89.2 (18.1)	referent	0.14	0.60
		AG	104	89.6 (19.8)	+1.52 (-3.07 to 6.12)		
		AA	40	87.8 (21.0)	+5.04 (-1.60 to 11.67)		
	rs10783219	AA	136	88.7 (19.4)	referent	0.44	0.39
		AT	116	89.8 (19.2)	-0.58 (-5.16 to 4.01)		
		TT	34	88.5 (16.5)	-2.61 (-9.30 to 4.08)		
	rs4516035	TT	123	88.3 (18.4)	referent	0.43	0.16
		CT	112	89.3 (19.9)	-2.24 (-7.12 to 2.64)		
		CC	51	91.3 (19.5)	-2.47 (-8.68 to 3.73)		
	rs11568820	CC	158	89.4 (19.1)	referent	0.21	0.51
		CT	82	91.8 (18.7)	+3.96 (-0.88 to 8.81)		
		TT	42	84.0 (19.1)	+4.38 (-2.43 to 11.19)		
	rs7976091	CC	159	89.3 (19.2)	referent	0.14	0.33
		CT	83	90.7 (18.9)	+3.65 (-1.18 to 8.47)		
		TT	41	84.7 (18.8)	+5.13 (-1.73 to 12.00)		
	rs2238136	CC	172	88.6 (19.1)	referent	0.64	0.53
		CT	97	90.2 (19.8)	-0.50 (-5.09 to 4.09)		
		TT	22	91.4 (16.1)	-1.93 (-10.09 to 6.23)		
	rs1544410	CC	101	89.6 (15.7)	referent	0.31	0.20
		CT	134	89.1 (21.0)	-0.31 (-4.96 to 4.34)		
		TT	52	90.2 (19.6)	+3.14 (-2.94 to 9.23)		
	rs2228570	GG	114	87.9 (19.3)	referent	0.25	0.090
		AG	132	88.9 (19.6)	+0.36 (-4.27 to 5.00)		
		AA	45	94.5 (16.4)	+3.76 (-2.68 to 10.20)		
	rs2853559	GG	126	88.1 (19.0)	referent	0.65	0.16
		AG	122	89.5 (19.0)	-1.54 (-6.20 to 3.11)		
		AA	41	91.1 (19.1)	-1.49 (-7.97 to 5.00)		
	rs7970314	AA	153	89.6 (18.4)	referent	0.20	0.80
		AG	85	90.3 (20.1)	+2.87 (-1.94 to 7.67)		
		GG	44	84.1 (18.6)	+4.42 (-2.38 to 11.22)		
	rs7975232	AA	90	90.8 (20.0)	referent	0.099	0.14
		AC	129	88.4 (19.7)	-2.98 (-7.78 to 1.82)		
		CC	60	86.8 (14.7)	-5.05 (-11.05 to 0.96)		

[1] *Adjusted for potential determinants of FEV_1: Sex, age, ethnicity, body mass index, socio-economic position, smoking status, alcohol consumption, influenza vaccination, pneumococcal vaccination, and baseline vitamin D status. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category. ** Genotype could not be analysed due to collinearity with predictor variable. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for trend or interaction between baseline vitamin D status and genotype remain significant.*

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, SNP: Single nucleotide polymorphism, SD: Standard deviation, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor A, VDR: Vitamin D receptor, CUBN: Cubilin.

Table 4.11. Genetic determinants of Forced Vital Capacity (FVC).

Gene	SNP	Genotype	N	Mean FVC (SD)	Multivariable model: beta coefficient (95% CI) [1]	P value for trend	P value for genotype* 25(OH)D interaction
CYP3A4	rs2740574	AA	249	1.05 (0.16)	referent	0.87	0.91
		AG	33	0.98 (0.14)	+0.01 (-0.05 to 0.07)		
		GG	10	0.86 (0.15)	+0.01 (-0.10 to 0.11)		
CUBILIN	rs3740165	TT	259	1.03 (0.16)	referent	-	-
		TC	29**	1.03 (0.16)	-		
		CC	0*	-	-		
RXRA	rs7861779	GG	182	1.06 (0.15)	referent	0.21	0.012
		GA	86	1.00 (0.15)	-0.03 (-0.07 to 0.01)		
		AA	17	0.91 (0.19)	-0.05 (-0.01 to 0.03)		
	rs9409929	GG	125	1.03 (0.15)	referent	0.90	0.94
		AG	127	1.04 (0.16)	-0.00 (-0.04 to 0.04)		
		AA	35	1.04 (0.18)	-0.00 (-0.06 to 0.05)		
CYP24A1	rs6013897	TT	174	1.04 (0.16)	referent	0.46	0.53
		AT	96	1.03 (0.16)	-0.01 (-0.04 to 0.03)		
		AA	14	1.01 (0.17)	-0.03 (-0.11 to 0.05)		
	rs2762934	GG	206	1.04 (0.16)	referent	0.34	0.15
		AG	77	1.02 (0.16)	-0.02 (-0.06 to 0.02)		
		AA	7	0.98 (0.20)	-0.05 (-0.16 to 0.06)		
	rs2762939	GG	159	1.06 (0.15)	referent	0.041	0.017
		CG	105	1.02 (0.16)	-0.02 (-0.05 to 0.02)		
		CC	22	0.96 (0.19)	-0.07 (-0.13 to -0.00)		
	rs2248137	CC	97	1.05 (0.15)	referent	0.61	0.44
		CG	129	1.04 (0.16)	-0.01 (-0.05 to 0.02)		
		GG	60	0.99 (0.17)	-0.01 (-0.06 to 0.04)		
DBP	rs16846876	AA	138	1.02 (0.17)	referent	0.11	0.55
		AT	127	1.04 (0.15)	-0.01 (-0.05 to 0.02)		
		TT	15	1.09 (0.10)	0.06 (-0.01 to 0.14)		
	rs7041	CC	91	1.07 (0.14)	referent	0.16	0.77
		AC	132	1.03 (0.17)	-0.02 (-0.06 to 0.02)		
		AA	63	0.97 (0.15)	-0.04 (-0.09 to 0.01)		
	rs12512631	TT	113	1.03 (0.15)	referent	0.54	0.58
		CT	137	1.03 (0.17)	+0.00 (-0.03 to 0.04)		
		CC	41	1.06 (0.15)	+0.02 (-0.04 to 0.07)		
	rs4588	GG	160	1.03 (0.16)	referent	0.17	0.93
		GT	113	1.03 (0.17)	-0.03 (-0.06 to 0.01)		
		TT	16	1.09 (0.11)	-0.05 (-0.02 to 0.13)		
	rs2070741	TT	240	1.04 (0.16)	referent	0.13	0.76
		TG	46	1.00 (0.14)	-0.02 (-0.06 to 0.03)		
		GG	1*	-	-		
	rs2298849	AA	175	1.03 (0.16)	referent	0.23	0.65
		AG	101	1.04 (0.16)	+0.01 (-0.03 to 0.05)		
		GG	12	1.03 (0.13)	+0.05 (-0.03 to 0.14)		
CYP27A1	rs17470271	AA	113	1.02 (0.16)	referent	0.79	0.95
		AT	133	1.04 (0.16)	-0.01 (-0.05 to 0.03)		
		TT	44	1.06 (0.16)	+0.01 (-0.04 to 0.06)		
CYP27B1	rs4646536	AA	132	1.03 (0.15)	referent	0.11	0.57
		AG	118	1.04 (0.16)	+0.02 (-0.02 to 0.05)		
		GG	30	1.01 (0.17)	-0.05 (-0.10 to 0.01)		
	rs4646537	TT	264	1.04 (0.16)	referent	0.17	0.88
		GT	26	1.02 (0.19)	+0.04 (-0.02 to 0.10)		
		GG	0*	-	-		
CYP2R1	rs10500804	TT	104	1.05 (0.16)	referent	0.10	0.062
		GT	143	1.02 (0.16)	-0.04 (-0.08 to -0.00)		
		GG	44	1.05 (0.16)	-0.04 (-0.10 to 0.01)		
	rs2060793	GG	107	1.02 (0.16)	referent	0.14	0.31

Table 4.11 continued.

Gene	SNP	Genotype	N	Mean FVC (SD)	Multivariable model: beta coefficient (95% CI) [1]	P value for trend	P value for genotype* 25(OH)D interaction
		AG	129	1.04 (0.16)	+0.01 (-0.03 to 0.04)		
		AA	41	1.05 (0.15)	+0.04 (-0.01 to 0.09)		
	rs10766197	GG	86	1.04 (0.17)	referent	0.033	0.24
		AG	143	1.03 (0.16)	-0.04 (-0.08 to 0.00)		
		AA	43	1.03 (0.15)	-0.06 (-0.11 to -0.00)		
LRP2	rs3755166	GG	101	1.03 (0.16)	referent	0.62	0.71
		AG	151	1.04 (0.16)	-0.01 (-0.05 to 0.02)		
		AA	38	1.04 (0.16	-0.01 (-0.07 to 0.04)		
DHCR7	rs12785878	TT	154	1.07 (0.15)	referent	0.29	0.85
		GT	98	1.03 (0.15)	-0.01 (-0.05 to 0.03)		
		GG	38	0.93 (0.18)	-0.03 (-0.09 to 0.03)		
	rs3829251	GG	220	1.04 (0.16)	referent	0.66	0.20
		AG	69	1.02 (0.17)	+0.01 (-0.04 to 0.05)		
		AA	2*	0.93 (0.08)	-		
VDR	rs731236	AA	113	1.03 (0.16)	referent	0.54	0.54
		AG	132	1.04 (0.16)	-0.00 (-0.04 to 0.04)		
		GG	42	1.05 (0.14)	+0.02 (-0.04 to 0.07)		
	rs4334089	GG	145	1.05 (0.15)	referent	0.22	0.90
		AG	104	1.03 (0.17)	+0.01 (-0.03 to 0.04)		
		AA	40	1.01 (0.17)	+0.03 (-0.02 to 0.09)		
	rs10783219	AA	136	1.03 (0.16)	referent	0.21	0.51
		AT	116	1.04 (0.16)	-0.00 (-0.04 to 0.03)		
		TT	34	1.02 (0.14)	-0.03 (-0.09 to 0.02)		
	rs4516035	TT	123	1.00 (0.16)	referent	0.92	0.96
		CT	112	1.05 (0.15)	+0.01 (-0.03 to 0.04)		
		CC	51	1.06 (0.16)	-0.00 (-0.05 to 0.05)		
	rs11568820	CC	158	1.05 (0.15)	referent	0.15	0.80
		CT	82	1.04 (0.16)	+0.02 (-0.02 to 0.06)		
		TT	42	0.98 (0.17)	+0.04 (-0.01 to 0.09)		
	rs7976091	CC	159	1.04 (0.16)	referent	0.11	0.72
		CT	83	1.03 (0.16)	+0.02 (-0.02 to 0.06)		
		TT	41	0.98 (0.18)	+0.05 (-0.01 to 0.10)		
	rs2238136	CC	172	1.03 (0.16)	referent	0.27	0.35
		CT	97	1.04 (0.16)	-0.01 (-0.04 to 0.03)		
		TT	22	1.04 (0.13)	-0.04 (-0.10 to 0.03)		
	rs1544410	CC	101	1.05 (0.15)	referent	0.81	0.35
		CT	134	1.02 (0.16)	-0.02 (-0.06 to 0.02)		
		TT	52	1.05 (0.18)	+0.01 (-0.04 to 0.06)		
	rs2228570	GG	114	1.01 (0.16)	referent	0.91	0.17
		AG	132	1.06 (0.16)	+0.04 (-0.00 to 0.08)		
		AA	45	1.03 (0.15)	-0.00 (-0.05 to 0.05)		
	rs2853559	GG	126	1.01 (0.16)	referent	0.58	0.76
		AG	122	1.05 (0.15)	-0.00 (-0.04 to 0.04)		
		AA	41	1.08 (0.17)	+0.01 (-0.04 to 0.07)		
	rs7970314	AA	153	1.05 (0.15)	referent	0.25	0.51
		AG	85	1.04 (0.17)	+0.03 (-0.01 to 0.07)		
		GG	44	0.97 (0.16)	+0.03 (-0.02 to 0.09)		
	rs7975232	AA	90	1.04 (0.17)	referent	0.59	0.39
		AC	129	1.02 (0.16)	-0.02 (0.06 to 0.19)		
		CC	60	1.04 (0.15)	-0.01 (-0.06 to 0.04)		

[1] *Adjusted for potential determinants of FVC: Sex, age, ethnicity, body mass index, socio-economic position, smoking status, alcohol consumption, influenza vaccination, pneumococcal vaccination, and baseline vitamin D status. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category. ** Genotype could not be analysed due to collinearity with predictor variable. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for trend or interaction between baseline vitamin D status and genotype remain significant.*

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, SNP: Single nucleotide polymorphism, SD: Standard deviation, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor A, VDR: Vitamin D receptor, CUBN: Cubilin.

Table 4.12. Genetic determinants of fractional exhaled nitric oxide (FeNO).

Gene	SNP	Genotype	N [1]	Median FeNO (IQR)	Multivariable model: antilog of beta coefficient (95% CI) [2]	P value for trend	P value for genotype* 25(OH)D interaction
CYP3A4	rs2740574	AA	247	30 (19 to 43)	referent	0.70	0.99
		AG	33	29 (21 to 41)	1.08 (0.84 to 1.39)		
		GG	9	31 (21 to 42)	1.10 (0.69 to 1.75)		
CUBILIN	rs3740165	TT	256	29 (19 to 42)	referent	-	-
		TC	29**	30 (20 to 43)	-		
		CC	0*	-	-		
RXRA	rs7861779	GG	180	29 (19.5 to 41.5)	referent	0.84	0.29
		GA	85	31 (20 to 42)	1.01 (0.86 to 1.19)		
		AA	17	25 (19 to 42)	0.97 (0.69 to 1.35)		
	rs9409929	GG	124	30 (19 to 43)	referent	0.55	0.92
		AG	125	30 (20 to 42)	1.03 (0.88 to 1.20)		
		AA	35	28 (20 to 41)	1.07 (0.85 to 1.35)		
CYP24A1	rs6013897	TT	171	28 (19 to 43)	referent	0.46	0.30
		AT	96	30 (20 to 42)	1.04 (0.89 to 1.21)		
		AA	14	34 (28 to 42)	1.13 (0.81 to 1.58)		
	rs2762934	GG	204	30 (20 to 43)	referent	0.54	0.93
		AG	76	28 (18 to 39.5)	0.87 (0.74 to 1.03)		
		AA	7	36 (19 to 76)	1.15 (0.73 to 1.83)		
	rs2762939	GG	159	30 (20 to 43)	referent	0.69	0.42
		CG	102	29 (17 to 42)	0.97 (0.83 to 1.13)		
		CC	22	25 (18 to 35)	0.94 (0.71 to 1.25)		
	rs2248137	CC	96	30.5 (20 to 46)	referent	0.80	0.75
		CG	128	28.5 (18 to 41)	0.95 (0.80 to 1.12)		
		GG	59	30 (19 to 42)	1.03 (0.83 to 1.27)		
DBP	rs16846876	AA	136	29 (19.5 to 41.5)	referent	0.42	0.70
		AT	126	30 (20 to 45)	1.04 (0.89 to 1.21)		
		TT	15	32 (18 to 58)	1.15 (0.82 to 1.59)		
	rs7041	CC	90	30 (20 to 42)	referent	0.53	0.54
		AC	131	29 (19 to 43)	1.03 (0.87 to 1.23)		
		AA	62	29.5 (19 to 41)	0.93 (0.76 to 1.15)		
	rs12512631	TT	113	30 (19 to 41)	referent	0.50	0.85
		CT	134	30 (20 to 45)	1.10 (0.95 to 1.29)		
		CC	41	28 (20 to 35)	0.93 (0.75 to 1.15)		
	rs4588	GG	158	29 (19 to 41)	referent	0.42	0.46
		GT	112	32.5 (21 to 47.5)	1.05 (0.90 to 1.23)		
		TT	16	21 (16 to 39)	0.88 (0.63 to 1.21)		
	rs2070741	TT	237	30 (19 to 43)	referent	0.26	0.97
		TG	46	30.5 (21 to 41)	1.02 (0.84 to 1.25)		
		GG	1*	-	-		
	rs2298849	AA	172	30 (19.5 to 42)	referent	0.006	0.17
		AG	101	30 (21 to 45)	1.05 (0.90 to 1.22)		
		GG	12	20.5 (12.5 to 24.5)	0.61 (0.42 to 0.87)		
CYP27A1	rs17470271	AA	112	29 (19 to 38)	referent	0.77	0.43
		AT	131	30 (20 to 47)	1.07 (0.91 to 1.26)		
		TT	44	26 (20 to 45.5)	1.03 (0.83 to 1.29)		
CYP27B1	rs4646536	AA	131	30 (20 to 42)	referent	0.30	0.38
		AG	117	31 (21 to 45)	1.13 (0.97 to 1.31)		
		GG	30	21 (17 to 32)	0.88 (0.69 to 1.12)		
	rs4646537	TT	262	29 (19 to 43)	referent	0.75	0.54
		GT	25	31 (23 to 39)	1.04 (0.80 to 1.36)		
		GG	0*	-	-		
CYP2R1	rs10500804	TT	103	28 (20 to 45)	referent	0.091	0.14
		GT	141	30 (18 to 41)	0.96 (0.82 to 1.13)		
		GG	44	31 (22 to 45)	1.21 (0.97 to 1.51)		
	rs2060793	GG	105	30 (20 to 41)	referent	0.36	0.30

Table 4.12 continued.

Gene	SNP	Genotype	N [1]	Median FeNO (IQR)	Multivariable model: antilog of beta coefficient (95% CI) [2]	P value for trend	P value for genotype* 25(OH)D interaction
		AG	128	29.5 (19 to 44.5)	1.00 (0.85 to 1.18)		
		AA	41	29 (20 to 42)	0.90 (0.72 to 1.13)		
	rs10766197	GG	85	29 (20 to 45)	referent	0.12	0.29
		AG	142	30 (18 to 37)	0.92 (0.78 to 1.09)		
		AA	42	31.5 (24 to 47)	1.20 (0.95 to 1.51)		
LRP2	rs3755166	GG	100	27 (19 to 39.5)	referent	0.71	0.12
		AG	149	30 (20 to 45)	1.18 (1.00 to 1.39)		
		AA	38	28 (14 to 36)	1.05 (0.83 to 1.32)		
DHCR7	rs12785878	TT	154	28 (19 to 41)	referent	0.14	0.27
		GT	96	31 (20 to 43)	1.07 (0.91 to 1.26)		
		GG	37	30 (21 to 48)	1.21 (0.94 to 1.56)		
	rs3829251	GG	219	28 (20 to 43)	referent	0.76	0.88
		AG	67	30 (17 to 42)	1.01 (0.85 to 1.21)		
		AA	2*	-	-		
VDR	rs731236	AA	111	30 (20 to 40)	referent	0.71	0.89
		AG	132	29 (19 to 43)	1.02 (0.87 to 1.19)		
		GG	41	29 (20 to 45)	1.04 (0.83 to 1.30)		
	rs4334089	GG	144	30 (20 to 44)	referent	0.36	0.76
		AG	102	27 (19 to 41)	0.92 (0.78 to 1.07)		
		AA	40	33.5 (20 to 42)	0.90 (0.72 to 1.13)		
	rs10783219	AA	134	29.5 (20 to 42)	referent	0.58	0.69
		AT	115	29 (19 to 43)	1.04 (0.89 to 1.21)		
		TT	34	30 (20 to 44)	1.07 (0.85 to 1.34)		
	rs4516035	TT	122	28.5 (19 to 42)	referent	0.56	0.98
		CT	111	30 (20 to 42)	1.08 (0.91 to 1.27)		
		CC	50	28 (18 to 44)	1.07 (0.86 to 1.32)		
	rs11568820	CC	156	30 (19 to 44)	referent	0.81	0.85
		CT	82	27.5 (18 to 37)	0.90 (0.77 to 1.07)		
		TT	41	32 (22 to 42)	0.97 (0.77 to 1.23)		
	rs7976091	CC	157	30 (20 to 44)	referent	0.64	0.81
		CT	83	28 (18 to 39)	0.91 (0.77 to 1.07)		
		TT	40	30.5 (20.5 to 41.5)	0.95 (0.75 to 1.20)		
	rs2238136	CC	171	29 (20 to 43)	referent	0.49	0.91
		CT	95	29 (18 to 44)	0.97 (0.83 to 1.14)		
		TT	22	30 (22 to 36)	1.10 (0.84 to 1.46)		
	rs1544410	CC	100	30 (20 to 40)	referent	0.97	0.96
		CT	133	30 (20 to 44)	0.99 (0.84 to 1.16)		
		TT	51	27 (19 to 45)	1.00 (0.81 to 1.24)		
	rs2228570	GG	113	30 (19 to 43)	referent	0.76	0.39
		AG	130	30.5 (21 to 42)	1.07 (0.91 to 1.25)		
		AA	45	27 (19 to 39)	0.97 (0.78 to 1.20)		
	rs2853559	GG	124	30 (20 to 41.5)	referent	0.13	0.25
		AG	121	28 (19 to 42)	0.93 (0.79 to 1.09)		
		AA	41	33 (21 to 47)	1.19 (0.95 to 1.48)		
	rs7970314	AA	151	30 (19 to 44)	referent	0.85	0.89
		AG	85	28 (18 to 37)	0.93 (0.79 to 1.10)		
		GG	43	35 (22 to 42)	0.98 (0.77 to 1.24)		
	rs7975232	AA	89	29 (20 to 45)	referent	0.81	0.37
		AC	128	28.5 (18 to 42)	0.94 (0.80 to 1.12)		
		CC	59	32 (22 to 41)	1.03 (0.83 to 1.27)		

[1] *Exhaled nitric oxide levels not recorded in n=3.* ***[2]*** *Adjusted for potential determinants of FeNO: Sex, age, ethnicity, body mass index, socio-economic position, smoking status, alcohol consumption, influenza vaccination, pneumococcal vaccination, and baseline vitamin D status. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category. ** Genotype could not be analysed due to collinearity with predictor variable. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for trend or interaction between baseline vitamin D status and genotype remain significant.*

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor A, VDR: Vitamin D receptor, CUBN: Cubilin.

Table 4.13. Genetic determinants of asthma control test score (ACT).

Gene	SNP	Genotype	N	Median ACT (IQR)	Multivariable model: antilog of beta coefficient (95% CI) [1]	P value for trend	P value for genotype* 25(OH)D interaction
CYP3A4	rs2740574	AA	249	20 (17 to 22)	referent	0.48	0.60
		AG	33	20 (17 to 22)	1.04 (0.95 to 1.15)		
		GG	10	16.5 (14 to 21)	0.94 (0.80 to 1.11)		
CUBILIN	rs3740165	TT	259	20 (17 to 22)	referent	-	-
		TC	29**	20 (15 to 21)	-		
		CC	0*	-	-		
RXRA	rs7861779	GG	182	20 (17 to 22)	referent	0.38	0.31
		GA	86	20 (16 to 22)	1.02 (0.96 to 1.09)		
		AA	17	20 (13 to 21)	0.95 (0.84 to 1.07)		
	rs9409929	GG	125	20 (17 to 22)	referent	0.63	0.27
		AG	127	20 (16 to 22)	0.98 (0.93 to 1.04)		
		AA	35	19 (16 to 23)	1.02 (0.94 to 1.11)		
CYP24A1	rs6013897	TT	174	20 (17 to 22)	referent	0.34	0.38
		AT	96	20 (17 to 22)	1.04 (0.98 to 1.10)		
		AA	14	17 (15 to 22)	0.94 (0.83 to 1.07)		
	rs2762934	GG	206	20 (17 to 22)	referent	0.28	0.95
		AG	77	20 (17 to 23)	1.04 (0.98 to 1.11)		
		AA	7	20 (16 to 22)	1.10 (0.93 to 1.30)		
	rs2762939	GG	159	20 (17 to 22)	referent	0.23	0.13
		CG	105	19 (17 to 22)	1.04 (0.99 to 1.10)		
		CC	22	18.5 (16 to 22)	0.94 (0.85 to 1.04)		
	rs2248137	CC	97	20 (17 to 22)	referent	0.29	0.88
		CG	129	19 (17 to 22)	1.00 (0.94 to 1.06)		
		GG	60	21 (16.5 to 22)	1.04 (0.96 to 1.12)		
DBP	rs16846876	AA	138	20 (16 to 22)	referent	0.91	0.35
		AT	127	20 (17 to 22)	1.02 (0.96 to 1.08)		
		TT	15	19 (16 to 23)	0.99 (0.88 to 1.12)		
	rs7041	CC	91	20 (18 to 22)	referent	0.30	0.66
		AC	132	20 (16 to 22)	1.01 (0.94 to 1.07)		
		AA	63	20 (16 to 21)	1.04 (0.96 to 1.13)		
	rs12512631	TT	113	20 (17 to 23)	referent	0.52	0.34
		CT	137	20 (16 to 22)	0.95 (0.90 to 1.00)		
		CC	41	20 (18 to 22)	0.97 (0.90 to 1.06)		
	rs4588	GG	160	20 (17 to 22)	referent	0.97	0.79
		GT	113	20 (17 to 22)	0.96 (0.91 to 1.02)		
		TT	16	20 (17 to 21.5)	1.00 (0.88 to 1.13)		
	rs2070741	TT	240	20 (17 to 22)	referent	0.73	0.82
		TG	46	20 (17 to 22)	1.04 (0.97 to 1.12)		
		GG	1*	-	-		
	rs2298849	AA	175	20 (17 to 22)	referent	0.18	0.46
		AG	101	20 (16 to 22)	1.02 (0.96 to 1.07)		
		GG	12	20.5 (18 to 22)	1.10 (0.96 to 1.25)		
CYP27A1	rs17470271	AA	113	20 (16 to 21)	referent	0.87	0.79
		AT	133	20 (17 to 22)	1.03 (0.97 to 1.09)		
		TT	44	20 (17.5 to 23)	1.01 (0.93 to 1.09)		
CYP27B1	rs4646536	AA	132	20 (17.5 to 22)	referent	0.18	0.51
		AG	118	18.5 (16 to 21)	0.94 (0.89 to 1.00)		
		GG	30	20 (17 to 22)	0.94 (0.86 to 1.03)		
	rs4646537	TT	264	20 (17 to 22)	referent	0.19	0.50
		GT	26	20 (17 to 21)	1.06 (0.97 to 1.17)		
		GG	0*	-	-		
CYP2R1	rs10500804	TT	104	20 (17 to 22)	referent	0.97	0.49
		GT	143	19 (16 to 22)	0.94 (0.89 to 1.00)		
		GG	44	21 (18.5 to 23)	1.00 (0.92 to 1.08)		
	rs2060793	GG	107	20 (17 to 22)	referent	0.64	0.36

Table 4.13 continued.

Gene	SNP	Genotype	N	Median ACT (IQR)	Multivariable model: antilog of beta coefficient (95% CI) [1]	P value for trend	P value for genotype* 25(OH)D interaction
		AG	129	20 (16 to 22)	1.02 (0.96 to 1.08)		
		AA	41	20 (18 to 22)	1.02 (0.94 to 1.11)		
	rs10766197	GG	86	20 (17 to 22)	referent	0.63	0.56
		AG	143	19 (16 to 22)	0.94 (0.89 to 1.01)		
		AA	43	20 (18 to 22)	0.98 (0.90 to 1.07)		
LRP2	rs3755166	GG	101	21 (17 to 22)	referent	0.10	0.50
		AG	151	20 (17 to 22)	1.00 (0.95 to 1.07)		
		AA	38	19.5 (15 to 20)	0.93 (0.85 to 1.01)		
DHCR7	rs12785878	TT	154	20 (18 to 22)	referent	0.28	0.41
		GT	98	20 (16 to 22)	0.96 (0.90 to 1.02)		
		GG	38	18 (14 to 21)	0.95 (0.87 to 1.04)		
	rs3829251	GG	220	20 (17 to 22)	referent	0.84	0.028
		AG	69	19 (15 to 21)	0.93 (0.87 to 0.99)		
		AA	2*	19 (18 to 20)	-		
VDR	rs731236	AA	113	20 (18 to 22)	referent	0.84	0.23
		AG	132	20 (17 to 22)	1.00 (0.95 to 1.06)		
		GG	42	19.5 (15 to 22)	1.01 (0.93 to 1.09)		
	rs4334089	GG	145	20 (17 to 22)	referent	0.16	0.33
		AG	104	20 (16 to 22)	1.00 (0.95 to 1.06)		
		AA	40	20 (18 to 22.5)	1.06 (0.98 to 1.15)		
	rs10783219	AA	136	20 (17.5 to 22)	referent	0.60	0.99
		AT	116	19 (16 to 22)	0.97 (0.91 to 1.02)		
		TT	34	20 (17 to 22)	0.98 (0.90 to 1.06)		
	rs4516035	TT	123	20 (16 to 22)	referent	0.62	0.48
		CT	112	20 (17 to 22)	0.98 (0.92 to 1.04)		
		CC	51	20 (18 to 22)	1.02 (0.94 to 1.10)		
	rs11568820	CC	158	20 (17 to 22)	referent	0.44	0.98
		CT	82	20 (16 to 22)	0.99 (0.93 to 1.05)		
		TT	42	20 (16 to 22)	1.03 (0.95 to 1.12)		
	rs7976091	CC	159	20 (17 to 22)	referent	0.35	0.80
		CT	83	20 (17 to 22)	1.01 (0.95 to 1.07)		
		TT	41	20 (16 to 22)	1.04 (0.96 to 1.14)		
	rs2238136	CC	172	20 (17 to 22)	referent	0.54	0.92
		CT	97	20 (17 to 22)	1.01 (0.95 to 1.07)		
		TT	22	17.5 (17 to 21)	0.97 (0.87 to 1.07)		
	rs1544410	CC	101	20 (18 to 22)	referent	0.32	0.12
		CT	134	19.5 (16 to 22)	0.96 (0.91 to 1.02)		
		TT	52	19.5 (15.5 to 22)	0.96 (0.89 to 1.04)		
	rs2228570	GG	114	20 (17 to 22)	referent	0.74	0.16
		AG	132	20 (16 to 22)	0.97 (0.92 to 1.03)		
		AA	45	20 (18 to 22)	0.99 (0.91 to 1.07)		
	rs2853559	GG	126	20 (17 to 22)	referent	0.98	0.66
		AG	122	20 (16 to 22)	0.97 (0.91 to 1.03)		
		AA	41	20 (18 to 22)	1.00 (0.92 to 1.09)		
	rs7970314	AA	153	20 (17 to 22)	referent	0.27	0.74
		AG	85	20 (17 to 22)	1.01 (0.95 to 1.07)		
		GG	44	20 (16 to 21.5)	1.05 (0.96 to 1.14)		
	rs7975232	AA	90	19.5 (15 to 22)	referent	0.71	0.90
		AC	129	20 (17 to 22)	1.05 (0.99 to 1.12)		
		CC	60	20 (18 to 22)	1.01 (0.94 to 1.10)		

[1] *Adjusted for potential determinants of asthma control test score: Sex, age, ethnicity, body mass index, socio-economic position, smoking status, alcohol consumption, influenza vaccination, pneumococcal vaccination, and baseline vitamin D status. * Genotype could not be analysed due to <5 participants. ** Genotype could not be analysed due to collinearity with predictor variable. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for trend or interaction between baseline vitamin D status and genotype remain significant.*

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor A, VDR: Vitamin D receptor, CUBN: Cubilin.

Table 4.14. Genetic determinants of inhaled corticosteroid requirement (ICS).

Gene	SNP	Genotype	N [1]	Median ICS requirement, µg (IQR)	Multivariable model: antilog of beta coefficient (95% CI) [2]	P value for trend	P value for genotype* 25(OH)D interaction
CYP3A4	rs2740574	AA	247	400 (200 to 800)	referent	0.62	0.71
		AG	32	400 (200 to 1000)	0.95 (0.70 to 1.29)		
		GG	10	400 (200 to 1600)	1.15 (0.67 to 1.96)		
CUBILIN	rs3740165	TT	256	400 (200 to 800)	referent	-	-
		TC	29**	400 (400 to 1000)	-		
		CC	0*	-	-		
RXRA	rs7861779	GG	180	400 (200 to 800)	referent	0.86	0.90
		GA	85	400 (200 to 800)	1.02 (0.84 to 1.24)		
		AA	17	400 (250 to 500)	1.03 (0.70 to 1.54)		
	rs9409929	GG	123	400 (300 to 800)	referent	0.33	0.81
		AG	126	400 (200 to 800)	0.97 (0.81 to 1.17)		
		AA	35	400 (200 to 600)	0.87 (0.66 to 1.15)		
CYP24A1	rs6013897	TT	172	400 (200 to 800)	referent	0.20	0.55
		AT	95	400 (300 to 800)	1.02 (0.85 to 1.23)		
		AA	14	550 (400 to 1000)	1.30 (0.87 to 1.93)		
	rs2762934	GG	204	400 (200 to 800)	referent	0.64	0.32
		AG	76	400 (250 to 800)	1.02 (0.84 to 1.24)		
		AA	7	400 (200 to 1000)	1.14 (0.66 to 1.99)		
	rs2762939	GG	157	400 (200 to 800)	referent	0.72	0.55
		CG	104	400 (300 to 1000)	1.05 (0.87 to 1.26)		
		CC	22	450 (200 to 800)	0.94 (0.68 to 1.31)		
	rs2248137	CC	96	400 (200 to 800)	referent	0.49	0.23
		CG	127	400 (200 to 600)	0.91 (0.75 to 1.11)		
		GG	60	500 (225 to 800)	1.09 (0.85 to 1.40)		
DBP	rs16846876	AA	136	400 (200 to 800)	referent	0.44	0.34
		AT	127	400 (250 to 600)	0.90 (0.75 to 1.08)		
		TT	15	400 (200 to 500)	0.86 (0.58 to 1.27)		
	rs7041	CC	91	400 (200 to 800)	referent	0.66	0.25
		AC	131	400 (200 to 800)	0.90 (0.74 to 1.10)		
		AA	61	400 (200 to 800)	0.95 (0.74 to 1.21)		
	rs12512631	TT	112	400 (200 to 800)	referent	0.92	0.94
		CT	135	400 (200 to 800)	1.06 (0.88 to 1.28)		
		CC	41	400 (200 to 800	1.01 (0.78 to 1.32)		
	rs4588	GG	158	400 (200 to 800)	referent	0.60	0.61
		GT	112	400 (200 to 550)	0.91 (0.75 to 1.10)		
		TT	16	400 (250 to 750)	0.90 (0.61 to 1.33)		
	rs2070741	TT	238	400 (200 to 800)	referent	0.25	0.46
		TG	46	400 (300 to 800)	1.15 (0.91 to 1.45)		
		GG	0*	-	-		
	rs2298849	AA	174	400 (250 to 800)	referent	0.90	0.42
		AG	100	400 (200 to 800)	0.97 (0.81 to 1.17)		
		GG	11	400 (200 to 1000)	1.03 (0.66 to 1.62)		
CYP27A1	rs17470271	AA	112	400 (200 to 800)	referent	0.47	0.59
		AT	132	400 (250 to 800)	1.09 (0.90 to 1.31)		
		TT	43	400 (200 to 500)	0.91 (0.70 to 1.18)		
CYP27B1	rs4646536	AA	131	400 (213.3 to 800)	referent	0.57	0.13
		AG	116	400 (200 to 800)	0.94 (0.78 to 1.13)		
		GG	30	400 (200 to 800)	1.09 (0.81 to 1.45)		
	rs4646537	TT	261	400 (200 to 800)	referent	0.30	0.26
		GT	26	400 (250 to 600)	0.85 (0.62 to 1.16)		
		GG	0*	-	-		
CYP2R1	rs10500804	TT	102	400 (200 to 800)	referent	0.29	0.75
		GT	142	400 (200 to 800)	0.89 (0.73 to 1.08)		
		GG	44	400 (200 to 500)	0.87 (0.66 to 1.13)		
	rs2060793	GG	106	400 (200 to 800)	referent	0.93	0.049

Table 4.14 continued.

Gene	SNP	Genotype	N [1]	Median ICS requirement, µg (IQR)	Multivariable model: antilog of beta coefficient (95% CI) [2]	P value for trend	P value for genotype* 25(OH)D interaction
		AG	128	400 (200 to 600)	0.87 (0.72 to 1.05)		
		AA	40	400 (200 to 800)	0.99 (0.76 to 1.29)		
	rs10766197	GG	85	400 (200 to 800)	referent	0.41	0.62
		AG	142	400 (250 to 800)	0.94 (0.77 to 1.16)		
		AA	43	400 (200 to 500)	0.89 (0.68 to 1.17)		
LRP2	rs3755166	GG	98	400 (213.3 to 800)	referent	0.80	0.56
		AG	151	400 (200 to 800)	0.98 (0.81 to 1.19)		
		AA	38	400 (400 to 800)	1.04 (0.78 to 1.38)		
DHCR7	rs12785878	TT	153	400 (200 to 600)	referent	0.81	0.056
		GT	97	400 (300 to 800)	1.06 (0.87 to 1.30)		
		GG	37	400 (200 to 800)	0.96 (0.71 to 1.31)		
	rs3829251	GG	219	400 (200 to 800)	referent	0.95	0.42
		AG	67	500 (375 to 800)	1.15 (0.93 to 1.42)		
		AA	2*	562.5 (125 to 1000)	-		
VDR	rs731236	AA	111	400 (200 to 800)	referent	0.95	0.67
		AG	132	400 (231.7 to 800)	1.04 (0.86 to 1.25)		
		GG	41	400 (400 to 800)	1.01 (0.77 to 1.31)		
	rs4334089	GG	144	400 (200 to 800)	referent	0.68	0.086
		AG	104	400 (200 to 800)	1.10 (0.91 to 1.33)		
		AA	38	400 (400 to 800)	1.06 (0.80 to 1.39)		
	rs10783219	AA	133	400 (200 to 800)	referent	0.23	0.040
		AT	116	400 (275 to 800)	1.05 (0.88 to 1.27)		
		TT	34	400 (200 to 500)	0.85 (0.65 to 1.11)		
	rs4516035	TT	122	400 (200 to 800)	referent	0.44	0.23
		CT	111	400 (300 to 800)	1.14 (0.94 to 1.39)		
		CC	50	400 (200 to 800)	1.10 (0.86 to 1.41)		
	rs11568820	CC	157	400 (200 to 800)	referent	0.94	0.55
		CT	81	400 (200 to 800)	0.97 (0.80 to 1.19)		
		TT	41	400 (250 to 800)	1.01 (0.76 to 1.34)		
	rs7976091	CC	158	400 (200 to 800)	referent	0.91	0.31
		CT	82	400 (200 to 600)	0.96 (0.79 to 1.17)		
		TT	40	400 (200 to 900)	0.98 (0.74 to 1.30)		
	rs2238136	CC	169	400 (200 to 800)	referent	0.090	0.10
		CT	97	400 (250 to 800)	1.00 (0.83 to 1.20)		
		TT	22	400 (200 to 400)	0.75 (0.54 to 1.04)		
	rs1544410	CC	99	400 (200 to 800)	referent	0.76	0.71
		CT	134	400 (200 to 800)	1.06 (0.8 to 1.28)		
		TT	51	400 (250 to 800)	1.04 (0.81 to 1.33)		
	rs2228570	GG	114	400 (300 to 800)	referent	0.030	0.42
		AG	129	400 (200 to 800)	0.93 (0.77 to 1.12)		
		AA	45	400 (200 to 500	0.75 (0.58 to 0.97)		
	rs2853559	GG	124	400 (200 to 600)	referent	0.96	0.18
		AG	122	400 (213.3 to 800)	1.23 (1.01 to 1.48)		
		AA	40	400 (200 to 800)	0.99 (0.76 to 1.30)		
	rs7970314	AA	152	400 (206.7 to 800)	referent	0.47	0.46
		AG	84	400 (200 to 1000)	0.91 (0.75 to 1.10)		
		GG	43	400 (200 to 1000)	1.11 (0.84 to 1.46)		
	rs7975232	AA	89	400 (200 to 800)	referent	0.27	0.51
		AC	129	400 (250 to 800)	1.06 (0.87 to 1.29)		
		CC	58	400 (200 to 600)	0.87 (0.67 to 1.12)		

[1] *ICS dose not recorded in n=3.* ***[2]*** *Adjusted for potential determinants of ICS requirement: Sex, age, ethnicity, body mass index, socio-economic position, smoking status, alcohol consumption, influenza vaccination, pneumococcal vaccination, and baseline vitamin D status.*

** Genotype could not be analysed due to <5 participants and/or too few events within this sub category. ** Genotype could not be analysed due to collinearity with predictor variable. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for trend or interaction between baseline vitamin D status and genotype remain significant.*

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor A, VDR: Vitamin D receptor, CUBN: Cubilin.

4.3. Discussion.

To my knowledge, this is the first study to investigate whether the influence of vitamin D status on asthma phenotype is modified by genetic variation in the vitamin D pathway. Vitamin D deficiency, defined using the 50 nmol/L 25(OH)D threshold, was present in the majority of participants, and it associated with classical environmental determinants of vitamin D status, but not with any potential genetic determinant investigated. No association was found between vitamin D status and a broad range of measures of asthma phenotype including symptom control, FEV1, FVC, ICS requirement and FeNO concentration. Neither did I find evidence to suggest that genetic variation in the vitamin D pathway influenced disease phenotype, either as a main effect, or in interaction with vitamin D status.

My findings with respect to prevalence and environmental determinants of vitamin D status are in keeping with those previously reported for other UK populations (298). By contrast, my finding of a lack of association between serum 25(OH)D concentrations and asthma phenotype - supported by the lack of association between vitamin D pathway SNP and asthma phenotype I observed - conflicts with the other studies in the literature, which have variously reported associations between lower vitamin D status and worse asthma control, more severe disease, lower FEV1 and increased requirement for inhaled corticosteroids.

Why might my findings differ? First, the majority of studies in the literature have investigated children with more severe asthma (245, 291-294), while our study is in adults with generally better symptom control. It may be that asthma phenotype is more readily modified by vitamin D in paediatric populations, and/or in those with more severe asthma: in keeping with the former hypothesis, randomised controlled trials of vitamin D supplementation to improve asthma control have tended to show protective effects in children (270, 299), but not in adults (209, 300). A second potential explanation is that residual confounding may have contributed to the findings of positive

associations in other studies: I collected detailed information on potential confounders of the relationship between vitamin D status and asthma control and adjusted for them in multivariable analyses. A third possibility is that publication bias may have contributed to the dearth of null studies in the published literature.

My analysis of other determinants of asthma phenotype identified a number of expected predictors, but also revealed some new information. The association between increased alcohol consumption and improved asthma control is intriguing: more research is needed to understand the relationship between alcohol intake and asthma control, and to identify potential mechanisms of protection if applicable. Our finding of an independent association between poorer asthma control and non-White ethnicity chimes with other reports (301, 302); the fact that serum 25(OH)D did not associate with ACT score in this analysis indicates that lower vitamin D status does not account for inter-ethnic differences in asthma control.

4.3.1. Study Strengths.

My study has several strengths. I investigated a wide range of potential environmental and genetic determinants of vitamin D status and asthma control, recorded detailed information on potential confounders of the relationship between 25(OH)D and asthma control, and phenotyped patients in considerable detail: spirometry and measurement of FeNO were performed using international guidelines and serum 25(OH)D concentrations were measured with the gold standard assay (LC-MS/MS) in a laboratory that participated in the international vitamin D external quality assurance scheme (www.deqas.org/). The study population included patients with mild, moderate and severe disease from both community and hospital settings, studied across all seasons: these features enhance generalisability of our results.

4.3.2. Study Limitations.

My study also has some limitations. A minority of participants had serum 25(OH)D concentrations >75 nmol/L, so I may have been underpowered to detect effects of the highest 25(OH)D concentrations on asthma phenotype: our results do not therefore definitively preclude beneficial effects of elevating serum 25(OH)D to >75 nmol/L. However, my findings are in keeping with clinical trials of vitamin D supplementation to improve asthma control in adults, which have been null to date (209, 300): however, lack of efficacy in these trials may reflect a lack of power, low prevalence of profound vitamin D deficiency at baseline, and / or sub-optimal dosing regimens. Meta-analysis of these trials is on-going and has potential to address the issue of type 2 error.

4.4. Conclusions.

In conclusion, I report that vitamin D deficiency is common in a UK adult population with ICS-treated asthma, and that this is influenced by the same classical environmental determinants of vitamin D status that operate in the general population. However, vitamin D status did not associate with genetic factors or with any one of a broad range of measures of asthma phenotype including symptom control, FEV_1, FVC, ICS requirement and FeNO concentration. Furthermore, genetic factors did not associate with measures of asthma phenotype directly, or in interaction with serum 25(OH)D concentration.

5. Cross-sectional analysis: Environmental and genetic determinants of vitamin D status among older adults in London, UK.

In this chapter I present a final cross-sectional analysis of factors, both environmental and genetic, which may associate with vitamin D status in a cohort of older adults living in sheltered housing (described in Methods, section 2.2.1) who attended a screening visit to participate in our ViDiFlu trial. This work was published online in January 2016 and is due to be published in print, in a vitamin D special issue of the Journal of Steroid Biochemistry and Molecular Biology which runs in Spring of 2016.

5.3. Introduction.

The risk of vitamin D deficiency is high in older adults due to the contribution of several physiological and lifestyle changes which occur with advancing age, such as a decrease in epidermal capacity to produce pre-vitamin D (303); an increase in the prevalence of chronic kidney disease (304) and a decline in sun-seeking behaviour due to avoidance or reduced mobility (305, 306). Besides these factors relating specifically to older age, several other environmental and genetic factors can further increase the risk of vitamin D deficiency in older adult populations: women often display lower 25-hydroxyvitamin D (25[OH]D) levels than men (307), possibly due to a greater adipose tissue component that sequesters the fat-soluble 25(OH)D compound from the circulation. Adipose sequestration may also be responsible for the inverse association often seen between body mass index (BMI) and 25(OH)D concentration (308, 309). Ethnicity and skin pigmentation affect 25(OH)D concentration in a number of ways: cutaneous vitamin D production depends on ultra-violet radiation (UVR) penetration which is limited by high skin melanin density (310), and ethnic variation in vitamin D pathway genes may also play a role (122). The level of cutaneous vitamin D synthesis may also be affected by lifestyle factors which affect UVR exposure, such as the amount of time spent

outdoors, the use of tanning beds, living or holidaying in sunny locations, dress-related skin exposure, and the use of sunscreen. Finally, several single nucleotide polymorphisms (SNP) in vitamin D pathway genes have been found to associate with serum 25(OH)D concentration. The most commonly known are within the vitamin D binding gene (*DBP*); 7-dehydrocholesterol reductase gene (*DHCR7*); and two cytochrome P450 enzyme genes (*CYP2R1* and *CYP24A1*) (122, 124).

Vitamin D deficiency is associated with increased susceptibility to several major causes of morbidity and mortality in older adults, including fractures (311), falls (312) and acute respiratory infections (231). It is known to be common among populations of older adults who are unable to mobilise outdoors, such as those in care homes (305, 306). However, there is relatively little data relating to vitamin D status of older adults in the UK who have better mobility, such as those living in sheltered accommodation. I therefore conducted a study to determine the prevalence of vitamin D deficiency in a cohort of older adults living in sheltered accommodation, and to identify environmental and genetic factors associating with low serum 25(OH)D concentration in this population.

5.4. Results.

5.4.1. Study population.

The population for this cross-sectional study was formed from the 222 older adult, sheltered accommodation residents screened between 29th March 2010 to 16th March 2012 for participation in the ViDiFlu trial (313). All screened respondents consented to anthropometric measurements; complete the lifestyle questionnaire; and donate blood samples for quantification of serum 25(OH)D concentration and for DNA storage and genotyping. Participant characteristics are presented in Table 6.1. Participants were aged 48-94 years at time of study induction; mean age was 72.0 years (SD 9.2). Females were more strongly represented in this study population (59.9%). The majority ethnic group was White (74.8%); 6.4% were Asian; 17.4% were Black; 1.4% had mixed ethnicity. Mean serum 25(OH)D concentration for all participants was 42.7 nmol/L (SD 22.0). 55 participants (24.8%) had serum 25(OH)D concentration <25 nmol/L; 89 (40.1%) had serum 25(OH)D concentration 25-49.9 nmol/L; 61 (27.5%) had serum 25(OH)D concentration 50-74.9 nmol/L; and 17 (7.7%) had serum 25(OH)D concentration ≥75 nmol/L.

Table 6.1. Participant Characteristics.

Factor	Category	N=222
Sex, n (%)	Female	133 (59.9)
	Male	89 (40.1)
Mean Age, yrs (SD)		72.0 (9.2)
Mean BMI, kg/m^2 (SD)		29.3 (6.8)
Ethnicity, n (%) [1]	White	163 (74.8)
	Asian / Asian British	14 (6.4)
	Black / Black British	38 (17.4)
	Mixed	3 (1.4)
Fitzpatrick Skin-type, n (%) [2]	1	19 (8.7)
	2	44 (20.2)
	3	72 (32.0)
	4	37 (17.0)
	5	26 (11.9)
	6	20 (9.2)
Socio-economic Position, n (%) [3]	1	72 (33.5)
	2	28 (13.0)
	3	14 (6.5)
	4	36 (16.7)
	5	62 (28.8)
	unclassified	3 (1.4)
Time outdoors, hours per day (%)[4]	>2hrs	82 (37.6)
	≤2hrs	136 (62.4)
Vitamin D supplement/day, n (%)[5]	Yes	55 (25.8)
	No	158 (74.2)
Quarter of blood draw, n (%)[6]	Q1 (Jan – Mar)	87 (39.4)
	Q2 (Apr – Jun)	59 (26.7)
	Q3 (Jul – Sep)	24 (10.9)
	Q4 (Oct – Dec)	51 (23.1)
Smoking status, n (%)	Non-current	187 (84.2)
	Current	35 (15.8)
Mean alcohol, units/month (SD)[7]		5.6 (12.7)
Recent sunny holiday, n (%) [8]	Yes	11 (5.0)
	No	208 (95.0)
Mean melanin skin density (SD) [9]		36.5 (12.8)
Hair loss, n (%) [10]	0	123 (55.4)
	1	39 (17.6)
	2	20 (9.0)
	3	36 (16.2)
	4	4 (1.8)
Serum 25(OH)D, nmol/L (%)	<25	55 (24.8)
	25 – 49.9	89 (40.1)
	50 – 74.9	61 (27.5)
	≥ 75	17 (7.6)
Mean serum 25(OH)D, nmol/L (SD)		42.7 (22.0)

[1] *Ethnicity not reported in n=4.* ***[2]*** *Fitzpatrick skin-type not reported in n=4. Classification of scores: 1 = extremely fair skin; always burn, never tan, 2 = fair skin; always burn, sometimes tan, 3 = pale skin; sometimes burn, always tan, 4 = olive skin; rarely burn, always tan, 5 = moderately pigmented brown skin; never burn, always tan, 6 = markedly pigmented black skin; never burn, always tan.* ***[3]*** *SEP not reported in n=7. Class definitions: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations.* ***[4]*** *Time outdoors not reported in n=4.* ***[5]*** *Supplementary vitamin D consumption not reported in n=9.* ***[6]*** *Quarter of blood draw not reported in n=1.* ***[7]*** *1 unit is defined as 10 millilitres of pure alcohol.* ***[8]*** *Recent sunny holiday not reported in n=3.Defined as a trip to any location within a latitude 51o North/South of the equator, during the local sunny season, 2 months prior to blood draw, for a duration of ≥1 week. †Adjusted for sex, ethnicity, vitamin D supplement consumption, quarter of blood draw, skin type and tanning bed use. [4] Skin melanin density: readings taken from inside left arm, measured in arbitrary units.* ***[9]*** *Skin melanin density: readings taken from inside left arm, measured in arbitrary units; range of values: 16.4 – 90.1.* ***[10]*** *Hair loss: categories 0-4 were defined by merging Male (Norwood-Hamilton [NH]) and Female (Ludwig [LG]) scales of hair loss: 0 = No hair loss, 1 = NH categories II & III (minor recession of frontal hairline; significant frontal loss/significant frontal regression + early hair loss from crown) merged with LG category I (thinning of hair from anterior crown with minimal widening of parting), 2 = NH categories IV & V (further frontal loss and enlargement of crown; further enlargement of crown and bridge begins to separate) merged with LG category II (evident thinning of crown), 3 = NH categories VI & VII (bridge disappears leaving large bald area on front and top of scalp; only back of scalp retains significant amount of hair) merged with LG category III (crown becomes denuded with significant parting, but hairline remains), 4 = other.*

Abbreviations: BMI: Body mass index, SD: Standard deviation, nmol/L: Nanomoles per litre.

5.4.2. Environmental determinants of vitamin D status.

The environmental determinants of vitamin D status are presented in Table 6.2. Multivariate analysis identified the following factors that independently associated with serum 25(OH)D concentration: Non-white ethnicity associated with a 8.6 nmol/L lower serum 25(OH)D concentration (95% CI -14.9 to -2.3, P=0.008). Lack of vitamin D supplement consumption associated with a 17.1 nmol/L lower serum 25(OH)D concentration (95% CI -23.3 to -10.9, P<0.001), referent to taking a daily dose. Referent to Q3/July-September sampling: Q1/January-March sampling associated with a 12.2 nmol/L lower serum 25(OH)D concentration (95% CI -21.5 to -2.9, P=0.01), and Q4/October-December sampling associated with a 10.3 nmol/L lower serum 25(OH)D concentration (95% CI -20.2 to -0.4, P=0.04). Sex, age, BMI, SEP, UVR exposure, skin-type, skin melanin density, smoking status, alcohol consumption, recent sunny holiday and hair loss did not independently associate with serum 25(OH)D concentration in this study population.

Table 6.2. Demographic & lifestyle determinants of serum 25-hydroxyvitamin D concentration.

Factor	Category	N	Mean 25(OH)D, nmol/L (SD)	Univariate P Value	Multivariable model – Beta Coefficient (95% CI)	P value†
Sex	Female	133	46.2 (21.6)	0.002	referent	
	Male	89	37.5 (21.6)		-6.95 (-14.2 to -0.33)	0.06
Age quartiles	1 (48.0 – 64.5)	55	40.5 (21.8)	0.07	referent	
	2 (64.6 – 71.8)	56	40.9 (22.5)		-1.64 (-9.24 to 5.96)	0.67
	3 (71.9 – 77.4)	55	49.7 (20.9)		+5.80 (-1.74 to 13.34)	0.13
	4 (77.5 – 94.1)	56	39.8 (21.9)		-3.96 (-11.62 to 3.69)	0.31
BMI, kg/m^2	<25	64	43.4 (22.7)	0.73	referent	
	≥25	158	42.4 (21.8)		-0.42 (-6.46 to 5.61)	0.89
Ethnicity [1]	White	163	44.5 (22.3)	0.03	referent	
	Other [2]	55	37.4 (20.9)		-8.57 (-14.86 to -2.27)	0.008
SEP [3]	1,2	100	45.2 (21.4)	0.18	referent	
	3,4,5	112	39.8 (22.3)		-2.64 (-8.37 to 3.09)	0.36
	unclassified	3	49.7 (30.9)		-3.90 (-27.25 to 19.46)	0.74
Time outdoors, hrs/day [4]	>2	82	44.2 (22.9)	0.42	referent	
	≤2	136	41.8 (21.7)		-1.42 (-7.02 to 4.18)	0.62
Vitamin D supplement [5]	Yes	55	56.5 (20.3)	<0.001	referent	
	No	158	38.6 (20.3)		-17.09 (-23.28 to -10.91)	<0.001
Quarter of blood draw [6]	Q1 (Jan – Mar)	87	37.1 (19.6)	<0.001	-12.20 (-21.50 to -2.89)	0.01
	Q2 (Apr – Jun)	59	48.7 (23.6)		-2.03 (-11.65 to 7.60)	0.68
	Q3 (Jul – Sep)	24	54.4 (21.7)		referent	
	Q4 (Oct – Dec)	51	39.3 (20.5)		-10.32 (-20.24 to -0.40)	0.04
Fitzpatrick skin-type [7]	1,2	63	42.5 (22.5)	0.19	-4.30 (-10.70 to -2.11)	0.19
	3,4	109	44.7 (22.2)		referent	
	5,6	46	38.1 (21.0)		-0.22 (-12.30 to 11.85)	0.97
Skin melanin density quartiles [8]	1	55	39.4 (22.7)	0.17	referent	
	2	65	45.3 (22.0)		+3.72 (-3.78 to 11.22)	0.33
	3	48	46.6 (20.9)		+5.84 (-2.33 to 14.01)	0.16
	4	54	39.5 (21.7)		+6.39 (-4.80 to 17.58)	0.26
Smoking status	Non-current	187	43.6 (22.5)	0.21	referent	
	Current	35	38.1 (18.8)		-5.14 (-12.93 to 2.65)	0.20
Alcohol consumption, units/wk	0	87	43.8 (21.5)	0.56	referent	
	1-20	121	41.5 (22.2)		-1.31 (-7.25 to 4.64)	0.67
	>20	14	46.5 (23.9)		+5.42 (-6.68 to 17.53)	0.38
Recent sunny holiday [9]	Yes	11	46.8 (18.8)	0.52	referent	
	No	208	42.5 (22.2)		-4.00 (-16.15 to 8.16)	0.52
Hair loss [10]	0	123	46.1 (21.2)	0.03	referent	
	1	39	40.3 (25.2)		+0.14 (-9.12 to 9.40)	0.98
	2	20	38.0 (17.9)		-3.51 (-14.90 to 7.89)	0.55
	3	36	34.9 (19.7)		-2.93 (-13.74 to 7.88)	0.59
	4	4	55.5 (32.0)		+14.23 (-8.82 to 37.28)	0.23

[1] *Ethnicity not reported in n=4.* ***[2]*** *Other ethnicities: n=14 Asian, n=38 Black, n=3 Mixed ethnicity.* ***[3]*** *SEP not reported in n=7. Class definitions: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations, 6 = student, 7 = Never/Long-term (>5yrs) unemployed.* ***[4]*** *UVR exposure not reported in n=4.* ***[5]*** *Supplementary vitamin D consumption not reported in n=9.* ***[6]*** *Quarter of blood draw not reported in n=1.* ***[7]*** *Fitzpatrick skin-type not reported in n=4. Classification of scores: 1 = extremely fair skin; always burn, never tan, 2 = fair skin; always burn, sometimes tan, 3 = pale skin; sometimes burn, always tan, 4 = olive skin; rarely burn, always tan, 5 = moderately pigmented brown skin; never burn, always tan, 6 = markedly pigmented black skin; never burn, always tan.* ***[8]*** *Skin melanin density: readings taken from inside left arm, measured in arbitrary units; range of values: 16.4 – 90.1.* ***[9]*** *Recent sunny holiday not reported in n=3. Defined as a trip to any location within a latitude 51^o North/South of the equator, during the local sunny season, 2 months prior to blood draw, for a duration of ≥1 week. †Adjusted for sex, ethnicity, vitamin D supplement consumption, quarter of blood draw, skin type and tanning bed use.* ***[10]*** *Hair loss: categories 0-4 were defined by merging Male (Norwood-Hamilton [NH]) and Female (Ludwig [LG]) scales of hair loss: 0 = No hair loss, 1 = NH categories II & III (minor recession of frontal hairline; significant frontal loss/significant frontal regression + early hair loss from crown) merged with LG category I (thinning of hair from anterior crown with minimal widening of parting), 2 = NH categories IV & V (further frontal loss and enlargement of crown; further enlargement of crown and bridge begins to separate) merged with LG category II (evident thinning of crown), 3 = NH categories VI & VII (bridge disappears leaving large bald area on front and top of scalp; only back of scalp retains significant amount of hair) merged with LG category III (crown becomes denuded with significant parting, but hairline remains), 4 = other.* ***†*** *Adjusted for sex, ethnicity, vitamin D supplement consumption, quarter of blood draw, and hair loss.*

Abbreviations: BMI: Body mass index, SEP: Socio-economic position, SD: Standard deviation, CI: Confidence interval.

5.4.3. Genetic determinants of vitamin D status.

Genetic determinants of vitamin D status are presented in table 6.3. After adjusting for significant lifestyle determinants of vitamin D status (ethnicity, vitamin D supplementation and season of sampling), one SNP in *DBP* independently associated with serum 25(OH)D concentration: AA genotype for rs7041 associated with 10.2 nmol/L lower level (95% CI –18.7 to -1.7), referent to CC genotype. After correcting for multiple comparison testing (Bejamini & Hochberg; false discovery rate of 5%) this association did not remain significant.

Table 6.3. Genetic determinants of serum 25-hydroxyvitamin D concentration.

Gene	SNP	Genotype	N	Mean 25(OH)D, nmol/L (SD)	Multivariable model – Beta Coefficient (95% CI)	P value [1] for trend
CYP24A1	rs6013897	TT	128	44.6 (23.3)	referent	0.39
		AT	75	40.7 (19.7)	-0.20 (-5.87 to 6.27)	
		AA	11	37.5 (23.5)	-5.87 (-18.13 to 7.05)	
	rs2248137	CC	72	44.2 (20.3)	referent	0.46
		CG	94	42.3 (24.1)	-1.03 (-7.46 to 5.41)	
		GG	51	40.5 (20.3)	-2.90 (-10.61 to 4.80)	
DBP	rs16846876	AA	118	43.1 (22.6)	referent	0.53
		AT	88	42.9 (21.7)	-3.01 (-8.79 to 2.77)	
		TT	15	40.9 (19.3)	-3.75 (-15.40 to 7.91)	
	rs7041	CC	58	48.4 (25.4)	referent	0.020
		AC	103	41.7 (21.2)	-4.84 (-11.55 to 1.88)	
		AA	53	37.8 (19.0)	-10.17 (-18.69 to -1.65)	
	rs12512631	TT	94	43.0 (21.8)	referent	0.69
		CT	101	41.7 (22.6)	-1.05 (-6.91 to 4.82)	
		CC	25	45.5 (21.8)	+1.94 (-7.60 to 11.48)	
	rs4588	GG	131	44.2 (22.5)	referent	0.19
		GT	81	41.0 (21.5)	-4.23 (-9.97 to 1.51)	
		TT	9	37.1 (19.4)	-9.67 (-24.29 to 4.95)	
	rs2070741	TT	181	43.3 (22.3)	referent	0.68
		TG	29	37.3 (20.2)	-6.21 (-14.34 to 1.93)	
		GG	2	44.0 (28.3)	-6.01 (-34.40 to 22.38)	
	rs2298849	AA	130	42.6 (21.9)	referent	0.42
		AG	69	44.2 (22.0)	+0.27 (-5.79 to 6.32)	
		GG	18	38.1 (24.5)	-4.28 (-14.74 to 6.18)	
CYP27B1	rs4646536	AA	103	44.9 (22.8)	referent	0.24
		AG	87	41.8 (21.1)	-3.09 (-8.98 to 2.80)	
		GG	27	39.3 (22.6)	-5.10 (-13.63 to 3.44)	
CYP2R1	rs10500804	TT	97	44.3 (22.3)	referent	0.46
		GT	96	40.5 (21.8)	-4.26 (-10.26 to 1.74)	
		GG	27	45.6 (22.3)	-3.48 (-12.72 to 5.76)	
	rs2060793	GG	67	41.5 (23.3)	referent	0.13
		AG	114	41.9 (21.4)	+1.62 (-4.73 to 7.97)	
		AA	38	47.9 (21.8)	+6.35 (-1.99 to 14.69)	
	rs10766197	GG	85	41.8 (21.5)	referent	0.78
		AG	94	41.9 (22.8)	+0.05 (-6.35 to 6.45)	
		AA	27	47.9 (23.1)	+1.37 (-8.23 to 10.97)	
DHCR7	rs12785878	TT	106	45.0 (21.9)	referent	0.52
		GT	79	42.4 (22.7)	-2.32 (-8.77 to 4.12)	
		GG	36	36.8 (20.1)	-3.04 (-12.40 to 6.31)	
	rs3829251	GG	155	42.7 (21.7)	referent	0.58
		AG	49	41.3 (23.0)	-0.88 (-7.62 to 5.85)	
		AA	8	46.0 (25.9)	+4.13 (-10.43 to 18.68)	
VDR	rs10783219	AA	97	40.2 (21.5)	referent	0.29
		AT	90	44.1 (21.6)	+3.64 (-2.61 to 9.88)	
		TT	27	45.3 (26.2)	+4.95 (-4.23 to 14.14)	

[1] *Adjusted for sex, age, BMI, ethnicity, SEP, hours outdoors, vitamin D supplement consumption, season of blood draw, Fitzpatrick skin-type, smoking status, alcohol consumption, recent sunny holiday, tanning bed use, hair loss, melanin density, and corrected for multiple comparisons testing, using the Benjamini & Hochberg method with a 5% false discovery rate.*

Abbreviations: SNP: Single nucleotide polymorphism, SD: Standard deviation, CI: Confidence interval, CYP-: Cytochrome P450-, DBP: vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase, VDR: vitamin D receptor.

5.5. Discussion.

To my knowledge this is the first study to comprehensively investigate the environmental and genetic determinants of vitamin D status in a UK cohort of older adults residing in sheltered accommodation (community housing). The majority of participants (64.9%) were vitamin D deficient at the 50 nmol/L 25(OH)D threshold; the mean serum 25(OH)D concentration for all participants was 42.7 nmol/L. These levels are comparable to those reported in a recent UK national survey for over 65 year olds living in private households (314), and higher than those reported among older adults in care home settings who are unable to mobilise out of doors (306, 315).

Three environmental factors independently associated with lower vitamin D status: non-white ethnicity, lack of vitamin D supplement consumption, and sampling in Winter or Spring. Non-White ethnicity (25% of the cohort) associated with a 8.6 nmol/L lower vitamin D status. Of the non-White study participants, the largest groups were Black / Black British (38/55 non-White participants), and Asian / Asian British (14/55 non-White participants). My findings agree with previous reports of vitamin D status in elderly participants: an observational study conducted in Baltimore, U.S. found lower serum 25(OH)D concentrations in African American participants aged ≥65 years, compared to White participants of a similar age (316), and one study conducted in Birmingham, UK reported a higher prevalence of vitamin D deficiency (57%) in elderly Asian participants as compared to elderly White participants (11%) (317). Consumption of daily vitamin D supplements associated with better vitamin D status: those taking a daily supplement had a 17.1 nmol/L higher 25(OH)D level, compared to those not taking a supplement. A cross-sectional study of elderly Dutch participants reported a similar association: from combined dietary sources of vitamin D they calculated that consumption of just 6.4 μg/day (256 IU) equated to a 16.8 nmol/L higher serum 25(OH)D concentration (318). My analysis also highlighted a statistically significant effect of season on serum 25(OH)D concentrations.

This finding is consistent with reports from two cross-sectional studies in UK adults (298, 319) where vitamin D status was reported to peak in September and to trough from January to March.

5.5.1. Study strengths.

This study has several strengths. A wide range of potential environmental and genetic determinants of vitamin D status were investigated in study participants that included objective measurement of skin pigmentation using a colorimeter and assessment of the extent of baldness. Two further strengths were the measurement of serum 25(OH)D concentration by LC-MS/MS in a laboratory participating in the DEQAS scheme, and the fact that the study was conducted over a 1 year period to allow observation of serum 25(OH)D concentrations across all seasons.

5.5.2. Study limitations.

The study also has some limitations. One limitation was the estimate of UVR exposure data by self-reported outdoor activity rather than by direct measurement of UVR exposure. Another limitation was the relatively small sample size used to investigate genetic determinants of vitamin D status – whilst the direction of association between SNP genotype and 25(OH)D concentrations in our study population were in agreement with those reported in previous studies, the paucity of statistically significant associations we identified may have arisen due to a lack of statistical power for detection, and the likelihood of type II error (false negatives/failure to reject the null hypothesis) was likely high owed to the strict false discovery rate (FDR) of 5% I set, using the Benjamini & Hochberg (BH) method. Setting a FDR threshold allows the researcher to control the expected proportion of type I errors (false positives/incorrect rejection of the null hypothesis) that exist within *significant* results. Whilst BH correction is a more forgiving, and arguably more sophisticated approach than the Bonferroni method that simply aims to eliminate type I error altogether by applying a cut-off threshold to all p values which consequently introduces a high degree of type II error, there is still the potential for

type II error with BH correction (320). The likelihood of this decreases with an increasing FDR (some use a 10%, 20%, or 25% threshold), but this tilts the balance back towards probable type I error.

There is no consensus in the field on the most appropriate FDR threshold to use, so in practice the compromise between acceptable levels of type I vs. type II error is often based upon the cost of discarding potentially true results. My approach could have been slightly less conservative by selecting an FDR of 10%, and this would have resulted in 3 SNP in the asthma cohort (Chapter 4) showing an independent association with vitamin D status after correcting for multiple comparisons, but that these SNP did not predict vitamin D status in the older adult and COPD study (Chapter 6) populations would suggest that type I error had occurred.

5.6. Conclusions.

In conclusion, I found that vitamin D deficiency was highly prevalent in a population of UK older adults living in sheltered accommodation, despite their ability to mobilise independently outdoors. Non-white ethnicity, lack of vitamin D supplement consumption and sampling in Winter and Spring independently associated with decreased serum 25(OH)D concentrations in this population, but genetic variation in the vitamin D pathway did not. The clinical implication of these findings is that consumption of vitamin D supplements is protective against vitamin D deficiency in this population, and vigorous efforts to improve uptake of such supplements should be made.

6. Cross-sectional analysis: Prevalence, determinants and clinical correlates of vitamin D deficiency in Chronic Obstructive Pulmonary Disease.

In this chapter I present a second cross-sectional analysis of lifestyle and genetic factors to associate with vitamin D status and the effect of vitamin D status on clinical markers of disease phenotype, on individuals diagnosed with COPD who attended a screening visit for participation in the ViDiCO trial (described in Methods, section 2.2.1).

6.1. Introduction.

Vitamin D deficiency has been reported to be more common in patients with chronic obstructive pulmonary disease (COPD) than in healthy controls (321-323), and has been found to associate with reduced forced expiratory volume in one second (FEV_1) / increased global initiative for chronic obstructive lung disease (GOLD) stage (323-328), reduced forced vital capacity (FVC), reduced peak expiratory flow (PEF), lower health-related quality of life (QoL) scores (324), as well as increased inhaled corticosteroid (ICS) requirement, and hypoxaemia (328). Reports of the association between vitamin D status and exercise capacity / skeletal muscle strength are conflicting, with one study reporting a positive association (326) and others reporting no associations (327, 329, 330).

To my knowledge, there are no previous cross-sectional investigations of serum 25(OH)D concentration and sputum biomarkers of COPD severity. Raised neutrophil and eosinophil levels and their inflammatory mediators have been identified during COPD exacerbation (331-333), and in a recent clinical trial we found vitamin D supplementation to significantly reduce risk of COPD exacerbation in those who were deficient at baseline (<50 nmol/L) (210). Furthermore, environmental and genetic factors which have been shown to influence vitamin D status in healthy populations (122, 334) have not yet been well characterised in COPD patients, nor have genetic factors which may associate with clinical markers of COPD phenotype.

I conducted a cross-sectional analysis to determine the prevalence of environmental and genetic determinants of serum 25(OH)D concentration in COPD patients being treated in London, UK, an whether associations exist between genetic variants in the vitamin D pathway and clinical correlates of COPD phenotype, specifically: % predicted FEV_1, % predicted forced vital capacity (FVC), FEV_1:FVC ratio, ICS requirement, Quality of Life ([Qol], as measured by the St. George's respiratory questionnaire), QS, and % eosinophils and neutrophils in induced sputum.

6.2. Results.

6.2.1. Study population.

A total of 278 adult COPD patients were enrolled in the study between 11th September 2009 and 12th April 2012. All consented to undergo clinical measurements and to donate blood samples for quantification of serum 25(OH)D and PTH concentration; 277/278 consented to donate a blood sample for DNA storage and genotyping. Participant characteristics are presented in Table 5.1. Age range was 40.8 to 91.9 years, with a mean of 66.4 years (SD 9.5). Most participants (60.8%) were male. The majority of participants (94.2%) classified their ethnic origin as being White; 1.8% were Asian/Asian British; 0.7% were Black/Black British; 1.8% were of mixed ethnicity; and 1.4% preferred not to report their ethnicity. Participants' COPD was managed exclusively in primary care in 202/278 (72.7%). Mean serum 25(OH)D concentration for all participants was 45.3 nmol/L (SD 25.4). Sixtyone participants (21.9%) had serum 25(OH)D concentration <25 nmol/L; 110 (39.6%) had serum 25(OH)D concentration in the range 25-49.9 nmol/L; 74 (26.6%) had serum 25(OH)D concentration in the range 50-74.9 nmol/L; and 33 (11.9%) had serum 25(OH)D concentration ≥75 nmol/L.

Table 5.1 Participant Characteristics.

Factor	Category	N=278
Sex, n (%)	Female	109 (39.2)
	Male	169 (60.8)
Mean Age, yrs (range)		66.4 (40.8 -91.9)
Mean BMI, kg/m^2 (SD)		27.7 (6.8)
Ethnicity, n (%) [1]	White	262 (94.2)
	Asian / Asian British	5 (1.8)
	Black / Black British	2 (0.6)
	Mixed	5 (1.8)
Fitzpatrick Skin-type, n (%) [2]	1	35 (12.8)
	2	56 (20.5)
	3	137 (50.2)
	4	29 (10.6)
	5	15 (5.5)
	6	1 (0.4)
Socio-economic Position, n (%) [3]	1	81 (29.7)
	2	24 (8.8)
	3	42 (15.4)
	4	62 (23.7)
	5	64 (23.4)
Time outdoors, hours/day (%) [4]	>2hrs	130 (47.6)
	≤2hrs	143 (52.4)
Daily vitamin D supplements, n (%) [5]	Any	52 (19.3)
	None	217 (80.7)
Quarter of sampling, n (%)	Q1 (Jan – Mar)	82 (29.5)
	Q2 (Apr – Jun)	55 (19.8)
	Q3 (Jul – Sep)	69 (24.8)
	Q4 (Oct – Dec)	72 (25.9)
Smoking status, n (%)	Non-current	169 (60.8)
	Current	109 (39.2)
Mean alcohol, units/week (SD) [6]		10.9 (18.3)
Managed exclusively in primary care, n (%)		202 (72.7)
Mean serum corrected calcium (SD) [7]		2.25 (0.09)
Recent sunny holiday, n (%) [8]	Yes	16 (5.9)
	No	257 (94.1)
Tanning bed use in previous yr, n (%) [9]	Yes	4
	No	269
Mean serum PTH (SD)		5.9 (3.0)
Serum PTH >6.8 pmol/L, n (%)	Yes	68 (24.5)
	No	210 (75.5)
Serum 25(OH)D, nmol/L (%)	≥75	33 (11.9)
	50 – 74.9	74 (26.6)
	25 – 49.9	110 (39.6)
	<25	61 (21.9)
Mean serum 25(OH)D (SD)		45.3 (25.4)

[1] *Ethnicity not reported in n=4. Mixed ethnicity participants: n=3 'White and Black Caribbean'; n=1 'White and Black African'; n=1 'White and Asian'.* ***[2]*** *Fitzpatrick skin-type score not reported in n=5. Categories defined as: 1 = extremely fair skin; always burn, never tan, 2 = fair skin; always burn, sometimes tan, 3 = pale skin; sometimes burn, always tan, 4 = olive skin; rarely burn, always tan, 5 = moderately pigmented brown skin; never burn, always tan, 6 = markedly pigmented black skin; never burn, always tan.* ***[3]*** *SEP not reported in n=5. Classes defined as: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations, 6 = student, 7 = Never/Long-term (>5yrs) unemployed.* ***[4]*** *Time outdoors not reported in n=5.* ***[5]*** *Vitamin D supplementation consumption not reported in n=9.* ***[6]*** *Alcohol consumption not reported in n=13.* ***[7]*** *Corrected calcium not measured in n=5.* ***[8]*** *Recent sunny holiday not reported in n=5. Defined as a trip to any location within a latitude 51^o North/South of the equator, during the local sunny season, 2 months prior to blood draw, for a duration of ≥1 week.* ***[9]*** *Tanning bed use not reported in n=5.*

Abbreviations: BMI: Body mass index, SD: Standard deviation, PTH: Parathyroid hormone, GOLD: Global initiative for chronic obstructive lung disease, nmol/L: Nanomoles per litre, pmol/L: Picomoles per litre, µg: Micrograms

6.2.2. Environmental determinants of serum 25(OH)D concentration.

Environmental determinants of vitamin D status are presented in Table 5.2. Multiple linear regression analysis showed the following factors to independently associate with lower serum 25(OH)D concentration: higher BMI (adjusted mean difference of 9.6 nmol/L for ≥25 kg/m^2 vs. <25 kg/m^2; 95% CI -15.7 to -3.4; P=0.002); lower SEP (adjusted mean difference of 6.2 nmol/L for higher vs. lower classes; 95% CI -12.4 to -0.0; P=0.049); lack of vitamin D supplement consumption (adjusted mean difference of 12.1 nmol/L for those taking any supplement vs. those taking no supplement; 95% CI -19.4 to -4.9; P=0.001); Sampling in January – March vs. July-September (adjusted mean difference 14.3nmol/L; 95% CI -22.6 to -6.0; P for trend = 0.005); and lack of a recent sunny holiday abroad (adjusted mean difference of 20.3nmol/L for those without vs. with such a holiday; 95% CI -32.9 to -7.7; P=0.002).

Table 5.2. Environmental determinants of serum 25-hydroxyvitamin D concentration in COPD patients.

Factor	Category	N	Serum 25(OH)D, nmol/L		Univariate P value [1]	Multivariable model - Beta Coefficient (95% CI)	P value [2]
			Mean (SD)	Mean difference			
Sex	Female	109	49.1 (29.2)	referent	0.049	referent	0.52
	Male	169	42.9 (22.3)	-6.2		-2.1 (-8.6 to 4.4)	
Age quartiles	1 (40.8 – 60.3 yrs)	69	45.3 (27.3)	referent	0.68	referent	0.84†
	2 (60.5 – 65.3 yrs)	70	47.1 (23.8)	+1.8		+1.5 (-6.9 to 9.9)	
	3 (65.3 – 72.3 yrs)	69	46.6 (28.3)	+1.3		0.0 (-8.5 to 8.5)	
	4 (72.4 – 91.9 yrs)	70	42.3 (21.9)	-3.0		-0.4 (-9.3 to 8.4)	
BMI, kg/m²	<25	109	48.9 (29.4)	referent	0.058	referent	0.001
	≥25	169	43.0 (22.1)	-5.9		-10.1 (-16.2 to -4.0)	
Ethnicity [3]	White	262	45.9 (25.7)	referent	0.29	referent	0.29
	Other	12	37.9 (17.2)	-8.0		-16.4 (-46.7 to 13.9)	
SEP [4]	Higher	105	50.0 (28.0)	referent	0.029	referent	0.037
	Lower	168	43.1 (23.2)	-6.9		-6.6 (-12.8 to -0.4)	
Time outdoors, hrs/day [5]	>2	130	50.5 (27.7)	referent	0.003	referent	0.069
	≤2	143	41.5 (22.2)	-9.0		-5.5 (-11.5 to 0.4)	
Vitamin D supplements [6]	Any	52	57.8 (27.8)	referent	<0.001	referent	<0.001
	None	217	42.8 (24.1)	-15.0		-12.4 (-19.6 to -5.2)	
Quarter of sampling	Q1 (Jan – Mar)	82	37.2 (23.4)	-15.2	0.001	-14.0 (-22.3 to -5.7)	0.006†
	Q2 (Apr – Jun)	55	43.7 (20.0)	-8.7		-8.0 (-16.9 to 0.8)	
	Q3 (Jul – Sep)	69	52.4 (24.5)	referent		referent	
	Q4 (Oct – Dec)	72	49.0 (29.5)	-3.4		-3.0 (-11.2 to 5.2)	
Fitzpatrick skin-type [7]	1,2	91	44.8 (24.7)	-1.9	0.73	-4.0 (-10.4 to 2.4)	0.42†
	3,4	166	46.7 (26.0)	referent		referent	
	5,6	16	42.3 (22.6)	-4.4		+7.2 (-19.7 to 34.0)	
Smoking status	Non-current	169	45.4 (22.2)	referent	0.97	referent	0.15
	Current	109	45.3 (29.8)	-0.1		-4.7 (-11.1 to 1.7)	
Alcohol consumption, units/wk [8]	0	121	47.4 (26.4)	referent	0.42	referent	0.49†
	1-20	98	44.1 (22.6)	-3.3		-5.3 (-12.0 to 1.4)	
	>20	46	42.4 (26.4)	-5.0		-2.9 (-11.2 to 5.5)	
Recent sunny holiday [9]	Yes	16	67.4 (36.0)	referent	<0.001	referent	0.002
	No	257	44.4 (24.0)	-23.0		-20.2 (-32.7 to -7.6)	
Tanning bed use, previous year [10]	Yes	4	90.8 (34.6)	referent	<0.001	*	
	No	269	45.1 (24.7)	-45.7			
GOLD Stage	I	69	51.4 (29.0)	referent	0.13	referent	0.015†
	II	131	43.3 (23.1)	-8.1		-10.4 (-17.8 to -3.0)	
	III	62	44.4 (26.2)	-7.0		-8.9 (-17.6 to -0.2)	
	IV	16	39.5 (20.2)	-11.9		-17.8 (-31.6 to -4.1)	

[1] *Univariate method: Student's T-test/One-way ANOVA.* ***[2]*** *Adjusted for all potential determinants of 25(OH)D concentration included in univariate analysis.* ***[3]*** *Ethnicity not reported in n=4.'Other' ethnicity defined as: n=5 Asian/Asian British, n=2 Black/Black British, n=5 mixed ethnicity.* ***[4]*** *SEP not reported in n=5. 'Higher' defined as classes: 1 (managerial, administrative and professional occupations) and 2 (intermediate occupations), 'Lower' defined as classes: 3 (small employers and own account workers), 4 (lower supervisory and technical occupations), and 5 (semi-routine and routine occupations).* ***[5]*** *Time outdoors not reported in n=5.* ***[6]*** *Vitamin D supplementation consumption not reported in n=9.* ***[7]*** *Fitzpatrick skin-type score not reported in n=5. Categories defined as: 1 = extremely fair skin; always burn, never tan, 2 = fair skin; always burn, sometimes tan, 3 = pale skin; sometimes burn, always tan, 4 = olive skin; rarely burn, always tan, 5 = moderately pigmented brown skin; never burn, always tan, 6 = markedly pigmented black skin; never burn, always tan.* ***[8]*** *Alcohol consumption not reported in n=13.* ***[9]*** *Recent sunny holiday not reported in n=5. Defined as a trip to any location within a latitude 51° North/South of the equator, during the local sunny season, 2 months prior to blood draw, for a duration of ≥1 week.* **†** *P value for trend.* ***** *Tanning bed use omitted from multivariable analysis due to <5 participants in 'yes' category.*

Abbreviations: BMI: Body mass index, SEP: Socio-economic position, GOLD: Global initiative for chronic obstructive lung disease, nmol/L: Nanomoles per litre, SD: Standard deviation, CI: Confidence interval

6.2.3. Genetic determinants of serum 25(OH)D concentration.

Genetic determinants of vitamin D status are presented in Table 5.3. After adjusting for sex, age, ethnicity, BMI, SEP, time outdoors, quarter of sampling, vitamin D supplement consumption, recent sunny holiday and GOLD stage, and correcting for multiple comparison testing (Benjamini & Hochberg, FDR=0.05) none of my investigated SNP independently associated with serum 25(OH)D concentration.

Table 5.3. Genetic determinants of serum 25-hydroxyvitamin D concentration in COPD patients.

Gene	SNP	Genotype	N [1]	Serum 25(OH)D, nmol/L		Multivariable model - Beta Coefficient (95% CI)	P Value for trend [2]
				Mean (SD)	Mean difference		
CYP24A1	rs6013897	TT	180	44.3 (25.5)	referent	referent	0.54
		AT	78	47.3 (25.2)	+3.0	+3.3 (-3.1 to 9.8)	
		AA	13	43.5 (21.1)	-0.8	+4.5 (-9.8 to 18.7)	
	rs2248137	CC	96	46.4 (29.4)	referent	referent	0.49
		CG	144	44.0 (23.1)	-2.4	+2.5 (-4.2 to 9.1)	
		GG	33	45.0 (21.7)	-1.6	+3.4 (-6.2 to 13.0)	
DBP	rs16846876	AA	124	47.2 (24.3)	referent	referent	0.41
		AT	126	43.3 (26.8)	-3.9	-2.6 (-8.9 to 3.6)	
		TT	23	42.2 (22.3)	-5.0	-2.2 (-13.2 to 8.8)	
	rs7041	GG	65	50.3 (28.8)	referent	referent	0.010
		TG	147	44.0 (25.5)	-6.3	-6.3 (-13.4 to 0.9)	
		TT	57	40.9 (19.7)	-9.4	-11.6 (-20.4 to -2.9)	
	rs12512631	TT	113	44.2 (23.4)	referent	referent	0.12
		CT	130	45.3 (26.6)	+1.1	+0.2 (-6.2 to 6.6)	
		CC	28	48.7 (26.0)	+4.5	+8.2 (-2.2 to 18.5)	
	rs4588	CC	130	48.5 (26.1)	referent	referent	0.099
		CA	123	42.9 (25.4)	-5.6	-5.1 (-11.2 to 1.1)	
		AA	23	39.9 (20.3)	-8.6	-9.2 (-20.1 to 1.7)	
	rs2070741	TT	237	44.7 (25.5)	referent	referent	0.84
		TG	31	49.8 (24.1)	+5.1	+0.4 (-8.8 to 9.6)	
		GG	3	53.3 (19.1)	+8.6	-2.9 (-30.9 to 25.1)	
	rs2298849	AA	181	44.2 (24.5)	referent	referent	0.38
		AG	87	47.1 (27.5)	+2.9	+2.5 (-3.8 to 8.9)	
		GG	7	41.7 (18.1)	-2.5	-8.0 (-25.8 to 9.8)	
CYP27B1	rs4646536	AA	111	44.0 (27.2)	referent	referent	0.65
		AG	128	45.7 (25.2)	+1.7	+0.3 (-6.2 to 6.7)	
		GG	29	47.6 (22.8)	+3.6	+2.4 (-8.0 to 12.7)	
CYP2R1	rs10500804	TT	95	46.6 (28.6)	referent	referent	0.49
		GT	132	45.9 (24.7)	-0.7	-2.6 (-9.2 to 4.1)	
		GG	47	40.7 (21.1)	-5.9	-3.0 (-11.6 to 5.5)	
	rs2060793	GG	95	42.9 (23.6)	referent	referent	0.99
		AG	129	48.1 (48.1)	+5.2	+6.1 (-0.5 to 12.7)	
		AA	49	42.6 (25.2)	-0.3	0.0 (-8.6 to 8.6)	
	rs10766197	GG	79	45.8 (27.8)	referent	referent	0.53
		AG	132	46.0 (24.1)	+0.2	-2.5 (-9.4 to 4.4)	
		AA	57	41.9 (27.8)	-3.9	-2.6 (-10.8 to 5.6)	
DHCR7	rs12785878	TT	171	45.5 (25.7)	referent	referent	0.33
		GT	87	45.4 (25.8)	-0.1	-1.6 (-7.9 to 4.7)	
		GG	15	39.3 (17.9)	-6.2	-7.5 (-22.4 to 7.5)	
	rs3829251	GG	210	45.8 (25.6)	referent	referent	0.22
		AG	54	45.1 (25.1)	-0.7	-1.7 (-8.8 to 5.4)	
		AA	7	31.9 (10.1)	-13.9	-12.0 (-31.5 to 7.4)	
VDR	rs10783219	AA	106	44.6 (24.0)	referent	referent	0.026
		AT	126	46.6 (25.5)	+2.0	-3.4 (-9.8 to 3.1)	
		TT	33	37.8 (23.5)	-6.8	-10.9 (-20.4 to -1.3)	

[1] *Genotyping not conducted in n=1.* ***[2]*** *Adjusted for sex, age, BMI, ethnicity, SEP, hours outdoors, vitamin D supplement consumption, season of blood draw, Fitzpatrick skin-type, smoking status, alcohol consumption, recent sunny holiday, tanning bed use, and GOLD stage, and corrected for multiple comparisons testing, using the Benjamini & Hochberg method with a 5% false discovery rate.*

Abbreviations: DBP: vitamin D binding protein, CYP-: cytochrome P450-, DHCR7: 7-dehydrocholesterol reductase, VDR: vitamin D receptor, SNP: single nucleotide polymorphism, SD: standard deviation, CI: confidence interval, nmol/L: Nanomoles per litre.

6.2.4. Association between vitamin D status and COPD phenotype.

After adjustment for potential environmental confounders, profound vitamin D deficiency was found to associate independently with reduced % predicted FEV_1 (Table 5.4, adjusted mean difference of 9.6% for those with a serum 25(OH)D concentration of <25 nmol/L vs. those with serum 25(OH)D concentration ≥75 nmol/L; 95% CI -18.8 to -0.4), though the P value for trend across all 4 subgroups of vitamin D status was only borderline significant (P=0.060). Vitamin D status also positively associated with % predicted FVC (Table 5.5, adjusted mean difference of 12.5% for those with serum 25(OH)D concentration of <25 nmol/L vs. those with serum 25(OH)D concentration ≥75 nmol/L; 95% CI -20.7 to -4.2; P value for trend across all 4 categories of vitamin D status = 0.003). I found no statistically significant association between vitamin D status and the other markers of COPD phenotype investigated, namely: FEV_1:FVC ratio (Table 5.6), quadriceps strength (Table 5.7), St. George's Respiratory Questionnaire score (Table 5.8), inhaled corticosteroid dose (Table 5.9), % eosinophils in induced sputum (Table 5.10), or % neutrophils in induced sputum (Table 5.11). Multiple linear regression analysis revealed several other factors to independently associate with various phenotypic features of COPD. Two measures of increased COPD severity, as indicated by lower % predicted FEV_1 and FEV_1:FVC, showed an association with a BMI <25kg/m^2 (P=0.003 and P<0.001, respectively). Male sex also associated with lower % predicted FEV_1 (P=0.018) and % predicted FVC (P<0.001). Older age associated with decreasing FEV_1:FVC ratio (P for trend = 0.016). Diminished quadriceps strength associated with older age (P for trend <0.001) and non-smoking status (P=0.015). Poorer respiratory quality of life, as indicated by lower SGRQ scores, associated with older age (P for trend = 0.017), and % eosinophils in blood positively associated with % eosinophils in induced sputum (P for trend = 0.003).

Table 5.4. Determinants of % predicted Forced Expiratory Volume in 1 second (FEV_1).

Factor	Category	N	Mean % predicted FEV_1 (SD)	Univariate P value [1]	Multivariable model – beta coefficient (95% CI)	P value [2]
Sex	Female	109	68.6 (20.2)	0.001	referent	0.029
	Male	169	60.4 (20.2)		-6.1 (-11.6 to -0.6)	
Age quartiles	1 (40.8 – 60.3 yrs)	69	66.8 (19.8)	0.24	referent	0.085†
	2 (60.5 – 65.3 yrs)	70	65.3 (19.2)		-3.0 (-10.3 to 4.2)	
	3 (65.3 – 72.3 yrs)	69	61.6 (22.5)		-7.2 (-14.6 to 0.2)	
	4 (72.4 – 91.9 yrs)	70	60.7 (20.3)		-5.5 (-13.0 to 1.9)	
BMI, kg/m²	<25	109	59.3 (20.8)	0.005	referent	0.004
	≥25	169	66.4 (19.9)		+7.6 (2.4 to 12.8)	
Ethnicity [3]	White	262	63.7 (20.7)	0.52	referent	0.57
	Other	12	59.8 (13.4)		-3.6 (-16.0 to 8.9)	
SEP [4]	Higher	105	68.0 (19.7)	0.007	referent	0.089
	Lower	168	61.1 (20.6)		-4.6 (-10.0 to 0.7)	
Smoking status	Non-current	169	63.3 (21.4)	0.75	referent	0.89
	Current	109	64.1 (19.1)		+0.4 (-5.1 to 5.8)	
Alcohol consumption, units/wk [5]	0	121	63.2 (21.0)	0.76	referent	0.26†
	1-20	98	65.2 (18.9)		+3.2 (-2.4 to 8.8)	
	>20	46	64.8 (22.3)		+4.1 (-3.1 to 11.3)	
Influenza vaccination	Yes	255	63.7 (20.6)	0.91	referent	0.71
	No	23	63.1 (19.9)		-1.9 (-11.7 to 7.9)	
Pneumonia vaccination	Yes	179	62.9 (20.1)	0.45	referent	0.35
	No	99	64.9 (21.4)		+2.6 (-2.9 to 8.2)	
Serum 25(OH)D, nmol/L	≥75	33	69.3 (19.4)	0.16	referent	0.060†
	74.9 – 50	74	63.6 (20.0)		-5.8 (-14.4 to 2.8)	
	49.9 – 25	110	64.3 (20.2)		-4.0 (-12.2 to 4.3)	
	<25	61	59.4 (21.9)		-9.6 (-18.8 to -0.4)	

[1] *Univariate analysis method: Student's T-test / One-way ANOVA test.* ***[2]*** *Adjusted for all potential determinants of FEV_1 investigated in univariate analysis.* ***[3]*** *Ethnicity not reported in n=4.'Other' ethnicity defined as: n=5 Asian/Asian British, n=2 Black/Black British, n=5 mixed ethnicity.****[4]*** *SEP not reported in n=5. 'Higher' defined as classes: 1 (managerial, administrative and professional occupations) and 2 (intermediate occupations), 'Lower' defined as classes: 3 (small employers and own account workers), 4 (lower supervisory and technical occupations), and 5 (semi-routine and routine occupations).* ***[5]*** *Alcohol consumption not reported in n=13.* **†** *P value for trend.*

Abbreviations: BMI: Body mass index, SEP: Socio-economic position, SD: standard deviation, CI: confidence interval, nmol/L: Nanomoles per litre.

Table 5.5. Determinants of forced vital capacity (FVC).

Factor	Category	N	Mean % predicted FVC (SD)	Univariate P value [1]	Multivariable model – beta coefficient (95% CI)	P value [2]
Sex	Female	109	104.0 (19.0)	<0.001	referent	<0.001
	Male	169	92.2 (17.8)		-11.14 (-16.07 to -6.21)	
Age quartiles	1 (40.8 – 60.3 yrs)	69	98.6 (19.3)	0.69	referent	0.52†
	2 (60.5 – 65.3 yrs)	70	97.8 (18.6)		-0.77 (-7.29 to 5.75)	
	3 (65.3 – 72.3 yrs)	69	95.1 (18.7)		-3.57 (-10.21 to 3.08)	
	4 (72.4 – 91.9 yrs)	70	95.9 (20.1)		-1.36 (-8.05 to 5.32)	
BMI, kg/m^2	<25	109	98.3 (19.7)	0.29	referent	0.40
	≥25	169	95.8 (18.8)		-2.00 (-6.64 to 2.64)	
Ethnicity [3]	White	262	97.5 (19.3)	0.009	referent	0.045
	Other	12	82.7 (9.8)		-11.41 (-22.58 to -0.24)	
SEP [4]	Higher	105	101.5 (19.3)	0.002	referent	0.12
	Lower	168	94.3 (18.6)		-3.80 (-8.59 to 0.98)	
Smoking status	Non-current	169	96.6 (19.4)	0.78	referent	0.60
	Current	109	97.2 (18.8)		-1.29 (-6.19 to 3.61)	
Alcohol consumption, units/wk [5]	0	121	97.2 (19.8)	0.89	referent	0.31†
	1-20	98	98.1 (17.5)		+3.45 (-1.56 to 8.46)	
	>20	46	96.7 (20.0)		+3.30 (-3.14 to 9.73)	
Influenza vaccination	Yes	255	96.8 (18.8)	0.94	referent	0.97
	No	23	96.5 (23.5)		-0.19 (-8.97 to 8.59)	
Pneumonia vaccination	Yes	179	97.1 (17.0)	0.75	referent	>0.99
	No	99	96.3 (22.7)		+0.02 (-4.95 to 4.98)	
Serum 25(OH)D, nmol/L	≥75	33	107.4 (18.4)	0.002	referent	0.003†
	74.9 – 50	74	98.2 (18.0)		-7.07 (-14.80 to 0.66)	
	49.9 – 25	110	95.4 (18.9)		-8.00 (-15.39 to -0.59)	
	<25	61	92.0 (19.5)		-12.46 (-20.70 to -4.21)	

[1] *Univariate analysis method: Students T-test / One-way ANOVA test.* ***[2]*** *Adjusted for all potential determinants of FVC investigated in univariate analysis.* ***[3]*** *Ethnicity not reported in n=4, 'Other' ethnicity defined as: n=5 Asian/Asian British, n=2 Black/Black British, n=5 mixed ethnicity.* ***[4]*** *SEP not reported in n=5. 'Higher' defined as classes: 1 (managerial, administrative and professional occupations) and 2 (intermediate occupations), 'Lower' defined as classes: 3 (small employers and own account workers), 4 (lower supervisory and technical occupations), and 5 (semi-routine and routine occupations).* ***[5]*** *Alcohol consumption not reported in n=13.* **†** *P value for trend.*

Abbreviations: BMI: Body mass index, SEP: Socio-economic position, SD: Standard deviation, CI: confidence interval, nmol/L: Nanomoles per litre.

Table 5.6. Determinants of forced expiratory volume in 1 second to forced vital capacity ratio (FEV1:FVC).

Factor	Category	N	Median FEV1:FVC (IQR)	Univariate P value [1]	Multivariable model – antilog of beta coefficient (95% CI)	P value [2]
Sex	Female	109	0.57 (0.47 to 0.65)	0.003	referent	0.069
	Male	169	0.51 (0.40 to 0.62)		0.93 (0.87 to 1.01)	
Age quartiles	1 (40.8 – 60.3 yrs)	69	0.58 (0.49 to 0.65)	0.014	referent	0.019†
	2 (60.5 – 65.3 yrs)	70	0.55 (0.45 to 0.63)		0.95 (0.86 to 1.04)	
	3 (65.3 – 72.3 yrs)	69	0.54 (0.39 to 0.62)		0.89 (0.80 to 0.98)	
	4 (72.4 – 91.9 yrs)	70	0.49 (0.35 to 0.60)		0.90 (0.81 to 1.00)	
BMI, kg/m^2	<25	109	0.48 (0.37 to 0.59)	<0.001	referent	<0.001
	≥25	169	0.57 (0.47 to 0.64)		1.17 (1.09 to 1.26)	
Ethnicity [3]	White	262	0.53 (0.43 to 0.63)	0.33	referent	0.32
	Other	12	0.59 (0.54 to 0.59)		1.09 (0.92 to 1.29)	
SEP [4]	Higher	105	0.56 (0.46 to 0.65)	0.032	referent	0.15
	Lower	168	0.52 (0.40 to 0.62)		0.95 (0.88 to 1.02)	
Smoking status	Non-current	169	0.54 (0.42 to 0.63)	0.59	referent	0.31
	Current	109	0.55 (0.45 to 0.63)		1.04 (0.97 to 1.12)	
Alcohol consumption, units/wk [5]	0	121	0.55 (0.43 to 0.63)	0.99	referent	0.54†
	1-20	98	0.55 (0.45 to 0.63)		1.03 (0.95 to 1.11)	
	>20	46	0.54 (0.42 to 0.62)		1.03 (0.94 to 1.14)	
Influenza vaccination	Yes	255	0.54 (0.43 to 0.63)	0.79	referent	0.70
	No	23	0.52 (0.46 to 0.65)		0.97 (0.85 to 1.11)	
Pneumonia vaccination	Yes	179	0.53 (0.42 to 0.63)	0.16	referent	0.18
	No	99	0.56 (0.46 to 0.63)		1.05 (0.98 to 1.13)	
Serum 25(OH)D, nmol/L	≥75	33	0.55 (0.43 to 0.64)	0.48	referent	0.58†
	74.9 – 50	74	0.52 (0.42 to 0.62)		0.96 (0.85 to 1.08)	
	49.9 – 25	110	0.56 (0.45 to 0.64)		1.01 (0.90 to 1.13)	
	<25	61	0.50 (0.42 to 0.61)		0.95 (0.84 to 1.07)	

[1] *Univariate analysis method:Mann-Whitney / Kruskal-Wallis tests.* ***[2]*** *Adjusted for all potential determinants of FEV:FVC included in univariate analysis.* ***[3]*** *Ethnicity not reported in n=4.'Other' ethnicity defined as: n=5 Asian/Asian British, n=2 Black/Black British, n=5 mixed ethnicity.* ***[4]*** *SEP not reported in n=5. 'Higher' defined as classes: 1 (managerial, administrative and professional occupations) and 2 (intermediate occupations), 'Lower' defined as classes: 3 (small employers and own account workers), 4 (lower supervisory and technical occupations), and 5 (semi-routine and routine occupations).* ***[5]*** *Alcohol consumption not reported in n=13.* **†** *P value for trend.*

Abbreviations: BMI: Body mass index, SEP: Socio-economic position, IQR: Interquartile range, CI: confidence interval, nmol/L: Nanomoles per litre.

Table 5.7. Determinants of Quadriceps Strength (QS).

Factor	Category	N [1]	Mean QS, kg (SD)	Univariate P value [2]	Multivariable model – beta coefficient (95% CI)	P value [3]
Sex	Female	46	25.1 (10.9)	<0.001	referent	<0.001
	Male	88	36.5 (11.1)		+13.63 (9.41 to 17.86)	
Age quartiles	1 (43.5 to 58.7)	33	37.2 (13.9)	0.001	referent	<0.001†
	2 (58.9 to 64.4)	34	35.2 (12.3)		-5.45 (-10.35 to -0.56)	
	3 (64.6 to 70.0)	32	26.2 (9.5)		-10.80 (-15.90 to -5.69)	
	4 (70.2 to 91.7)	35	31.5 (10.5)		-9.25 (-14.69 to -3.81)	
BMI, kg/m^2	<25	68	32.2 (13.3)	0.73	referent	0.058
	≥25	66	32.9 (11.2)		+2.91 (-0.87 to 6.68)	
SEP [4]	Higher	50	31.8 (13.7)	0.60	referent	0.14
	Lower	84	33.0 (11.4)		-3.52 (-7.42 to 0.38)	
Smoking status	Non-current	70	31.4 (11.3)	0.23	referent	0.015
	Current	64	33.9 (13.2)		+4.65 (0.08 to 8.47)	
Alcohol consumption, units/wk [5]	0	53	30.4 (11.7)	0.036	referent	0.73†
	1-20	49	36.3 (12.2)		1.99 (-2.15 to 6.13)	
	>20	26	30.4 (13.3)		-1.38 (-6.23 to 3.48)	
Serum 25(OH)D, nmol/L	≥ 75	24	27.8 (11.8)	0.045	referent	0.803†
	74.9 – 50	36	36.8 (13.8)		+4.52 (-1.12 to 10.17)	
	49.9 – 25	51	32.0 (11.1)		+1.54 (-3.82 to 6.89)	
	<25	23	32.3 (11.2)		+0.72 (-5.55 to 6.98)	

[1] *Quadriceps strength measured in a subset of n=134.* ***[2]*** *Univariate analysis method: Students T-test / One-way ANOVA test.* ***[3]*** *Adjusted for all potential determinants of QS included in univariate analysis.* ***[4]*** *SEP not reported in n=5. 'Higher' defined as classes: 1 (managerial, administrative and professional occupations) and 2 (intermediate occupations), 'Lower' defined as classes: 3 (small employers and own account workers), 4 (lower supervisory and technical occupations), and 5 (semi-routine and routine occupations).* ***[5]*** *Alcohol consumption not reported in n=6.* **†** *P value for trend.*

Abbreviations: BMI: Body mass index, SEP: Socio-economic position, SD: Standard deviation, CI: confidence interval, nmol/L: Nanomoles per litre.

Table 5.8. Determinants of St. George's Respiratory Questionnaire score (SGRQ).

Factor	Category	N [1]	Mean SGRQ (SD)	Univariate P value [2]	Multivariable model – beta coefficient (95% CI)	P value [3]
Sex	Female	108	48.6 (17.5)	0.19	referent	0.30
	Male	167	45.6 (19.5)		-2.7 (-7.8 to 2.4)	
Age quartiles	1 (40.8 – 60.3 yrs)	69	52.5 (18.8)	0.030	referent	0.013†
	2 (60.5 – 65.3 yrs)	70	45.5 (19.5)		-7.3 (-14.1 to -0.6)	
	3 (65.3 – 72.3 yrs)	67	43.9 (18.8)		-9.0 (-15.8 to -2.1)	
	4 (72.4 – 91.9 yrs)	69	45.1 (17.0)		-8.7 (15.6 to -1.8)	
BMI, kg/m^2	<25	109	46.3 (19.2)	0.72	referent	0.66
	≥25	166	47.1 (18.5)		+1.1 (-3.7 to 5.9)	
Ethnicity [4]	White	260	47.0 (18.9)	0.58	referent	0.53
	Other	12	44.0 (16.0)		-3.7 (-15.2 to 7.8)	
SEP [5]	Higher	105	44.1 (17.5)	0.077	referent	0.10
	Lower	167	48.2 (19.3)		+4.1 (-0.8 to 9.0)	
Smoking status	Non-current	167	47.0 (18.5)	0.83	referent	0.16
	Current	108	46.4 (19.3)		-3.6 (-8.7 to 1.4)	
Alcohol consumption, units/wk [6]	0	119	49.1 (19.2)	0.034	referent	0.74†
	1-20	98	42.6 (18.3)		-6.1 (-11.2 to -0.9)	
	>20	46	47.6 (17.4)		-1.1 (-7.7 to 5.5)	
Influenza vaccination	Yes	254	46.6 (18.7)	0.53	referent	0.52
	No	21	49.2 (19.5)		+2.9 (-6.1 to 12.0)	
Pneumonia vaccination	Yes	177	47.8 (19.1)	0.21	referent	0.12
	No	98	44.8 (18.1)		-4.0 (-9.2 to 1.1)	
Serum 25(OH)D, nmol/L	≥75	33	44.1 (18.2)	0.56	referent	0.31†
	74.9 – 50	74	45.8 (18.4)		+2.3 (-5.7 to 10.3)	
	49.9 – 25	109	46.7 (18.8)		+2.5 (-5.2 to 10.1)	
	<25	59	49.4 (19.7)		+4.4 (-4.1 to 12.9)	

[1] *SGRQ completed by n=275.* ***[2]*** *Univariate analysis method: Students T-test / One-way ANOVA test.* ***[3]*** *Adjusted for all potential determinants of SGRQ included in univariate analysis.* ***[4]*** *Ethnicity not reported in n=3. 'Other' ethnicity defined as: n=5 Asian/Asian British, n=2 Black/Black British, n=5 mixed-ethnicity.* ***[5]*** *SEP not reported in n=3. 'Higher' defined as classes: 1 (managerial, administrative and professional occupations) and 2 (intermediate occupations), 'Lower' defined as classes: 3 (small employers and own account workers), 4 (lower supervisory and technical occupations), and 5 (semi-routine and routine occupations).* ***[6]*** *Alcohol consumption not reported in n=12.*
† *P value for trend.*

Abbreviations: BMI: Body mass index, SEP: Socio-economic position, SD: Standard deviation, CI: confidence interval, nmol/L: Nanomoles per litre.

Table 5.9. Determinants of Inhaled Corticosteroid (ICS) dose.

Factor	Category	N	Median ICS dose (IQR)	Univariate P value [1]	Multivariable model – antilog of beta coefficient (95% CI)	P value [2]
Sex	Female	109	500 (0 to 1000)	0.88	referent	0.84
	Male	169	400 (0 to 1000)		1.03 (0.79 to 1.34)	
Age quartiles	1 (40.8 – 60.3 yrs)	69	400 (0 to 1000)	0.43	referent	0.33†
	2 (60.5 – 65.3 yrs)	70	500 (0 to 1000)		1.32 (0.92 to 1.87)	
	3 (65.3 – 72.3 yrs)	69	400 (0 to 1000)		1.23 (0.86 to 1.76)	
	4 (72.4 – 91.9 yrs)	70	400 (0 to 1000)		1.24 (0.86 to 1.78)	
BMI, kg/m^2	<25	109	400 (0 to 1000)	0.71	referent	0.61
	≥25	169	500 (0 to 1000)		0.94 (0.72 to 1.21)	
Ethnicity [3]	White	262	480 (0 to 1000)	0.19	referent	0.83
	Other	12	900 (0 to 1500)		1.07 (0.57 to 2.00)	
SEP [4]	Higher	105	400 (0 to 1000)	0.57	referent	0.30
	Lower	168	500 (0 to 1000)		1.14 (0.89 to 1.48)	
Smoking status	Non-current	169	500 (0 to 1000)	0.95	referent	0.56
	Current	109	400 (0 to 1000)		1.09 (0.83 to 1.43)	
Alcohol consumption, units/wk [5]	0	121	400 (0 to 1000)	0.71	referent	0.25†
	1-20	98	450 (0 to 1000)		0.90 (0.68 to 1.18)	
	>20	46	490 (0 to 1000)		0.82 (0.59 to 1.15)	
Influenza vaccination	Yes	255	480 (0 to 1000)	0.65	referent	0.43
	No	23	400 (0 to 1000)		1.21 (0.75 to 1.93)	
Pneumonia vaccination	Yes	179	500 (0 to 1000)	0.83	referent	0.98
	No	99	400 (0 to 1000)		1.00 (0.77 to 1.31)	
Serum 25(OH)D, nmol/L	≥75	61	500 (0 to 1000)	0.85	referent	0.95†
	74.9 – 50	110	500 (0 to 1000)		1.04 (0.67 to 1.63)	
	49.9 – 25	74	450 (0 to 1000)		1.09 (0.72 to 1.67)	
	<25	33	200 (0 to 1000)		0.97 (0.60 to 1.56)	

[1] *Univariate analysis method: Mann-Whitney / Kruskal-Wallis tests.* ***[2]*** *Adjusted for all potential determinants of ICS score included in univariate analysis.* ***[3]*** *Other ethnicities: n=4 Asian, n=2 Black, n=5 Mixed-ethnicity, n=17 Undefined.* ***[4]*** *SEP not reported in n=5. 'Higher' defined as classes: 1 (managerial, administrative and professional occupations) and 2 (intermediate occupations), 'Lower' defined as classes: 3 (small employers and own account workers), 4 (lower supervisory and technical occupations), and 5 (semi-routine and routine occupations).* ***[5]*** *Alcohol consumption not reported in n=13.* **†** *P value for trend.*

Abbreviations: BMI: Body mass index, SEP: Socio-economic position, IQR: Interquartile range, CI: confidence interval, nmol/L: Nanomoles per litre.

Table 5.10. Determinants of sputum eosinophilia (subset of n=44 participants).

Factor	Category	N	Median eosinophilia (IQR)	Univariate P value [1]	Multivariable model – antilog of beta coefficient (95% CI)	P value [2]
Sex	Female	16	1.5 (0.5 to 3.2)	0.97	1.12 (0.63 to 2.00)	0.69
	Male	28	1.6 (0.5 to 6.8)		referent	
Age quartiles	1 (54.0 – 62.5)	11	0.8 (0.3 to 3.6)	0.64	referent	0.082†
	2 (62.6 – 68.2)	11	1.4 (0.6 to 9.4)		1.07 (0.46 to 2.51)	
	3 (68.3 – 71.6)	11	2.5 (0.4 to 3.0)		1.17 (0.52 to 2.56)	
	4 (71.7 – 91.7)	11	1.8 (0.7 to 13.6)		1.95 (0.92 to 4.13)	
BMI, kg/m^2	<25	9	1.6 (0.4 to 3.6)	0.87	referent	0.41
	≥25	35	1.5 (0.5 to 5.8)		0.75 (0.37 to 1.52)	
Blood eosinophilia	<3%	25	0.8 (0.3 to 2.5)	0.007	referent	0.003
	≥3%	19	3.3 (0.8 to 11.1)		2.68 (1.44 to 4.99)	
FEV_1:FVC	<0.58	22	1.5 (0.5 to 3.0)	0.66	referent	0.99
	≥0.58	22	1.7 (0.4 to 10.0)		1.00 (0.55 to 1.80)	
Serum 25(OH)D, nmol/L	<50	29	1.6 (0.5 to 5.8)	0.84	0.59 (0.28 to 1.25)	0.16
	≥50	15	1.5 (0.4 to 3.6)		referent	

[1] *Univariate analysis method: Mann-Whitney/Kruskal-Wallis tests.* ***[2]*** *Adjusted for all potential determinants of sputum eosinophilia investigated in univariate analysis.* **†** *P value for trend.*

Abbreviations: BMI: Body mass index, FEV_1:FVC: Forced expiratory volume in 1 second to forced vital capacity ratio, IQR: Interquartile range, CI: confidence interval, nmol/L: Nanomoles per litre.

Table 5.11. Determinants of sputum neutrophilia (subset of n=44 participants).

Factor	Category	N	Median neutrophilia (IQR)	Univariate P value [1]	Multivariable model – antilog of beta coefficient (95% CI)	P value [2]
Sex	Female	16	74.8 (66.8 to 76.8)	0.93	+0.1 (-7.5 to 7.8)	0.97
	Male	28	72.5 (65.3 to 81.5)		referent	
Age quartiles	1 (54.0 – 62.5)	11	75.4 (60.3 to 80.6)	0.42	referent	0.61†
	2 (62.6 – 68.2)	11	71.3 (68.1 to 74.2)		+4.5 (-7.5 to 16.4)	
	3 (68.3 – 71.6)	11	76.6 (72.3 to 83.0)		+8.9 (-2.5 to 20.3)	
	4 (71.7 – 91.7)	11	72.0 (62.3 to 82.4)		+1.5 (-9.6 to 12.5)	
Blood neutrophilia (%)	<60	21	71.3 (62.3 to 76.6)	0.37	referent	0.61
	≥60	23	75.4 (68.3 to 83.0)		+1.9 (-5.6 to 9.4)	
Predicted FEV_1(%)	<67.0	22	73.8 (68.1 to 79.9)	0.42	referent	0.75
	≥67.0	22	71.8 (65.4 to 80.6)		-1.2 (-9.1 to 6.6)	
Serum 25(OH)D, nmol/L	<50	29	72.4 (65.4 to 80.6)	0.66	+4.4 (-4.9 to 13.6)	0.34
	≥50	15	75.4 (70.5 to 79.9)		referent	

[1] *Univariate analysis method: Students T-Test/One-way ANOVA.* ***[2]*** *Adjusted for all potential determinants of sputum eosinophilia investigated in univariate analysis.* **†** *P value for trend.*

Abbreviations: FEV_1: Forced expiratory volume in 1 second, IQR: Interquartile range, CI: confidence interval, nmol/L: Nanomoles per litre.

6.2.5. Association between genetic factors and COPD phenotype.

Genetic determinants of clinical correlates of COPD phenotype are presented in Table 5.12 (FEV_1), Table 5.13 (FVC), Table 5.14 (FEV_1:FVC), and Table 5.15 (QS). After correcting for multiple comparisons testing (Benjamini & Hochberg method with a 5% false discovery rate) none of the genetic factors which independently associated with markers of COPD phenotype as main effects, or by interaction with baseline vitamin D status, remained significant.

Table 5.12. Genetic determinants of % predicted forced expiratory volume in 1 second (ppFEV$_1$).

Gene	SNP	Genotype	N	Mean FEV$_1$ (SD)	Multivariable model: beta coefficient (95% CI) [1]	P value for trend	P value for genotype* 25(OH)D interaction
CYP3A4	rs2740574	AA	247	64.1 (20.8)	referent	0.64	0.51
		AG	26	59.8 (17.0)	-5.36 (-14 to 3.35)		
		GG	1*	-	9.37 (-30.3 to 49.1)		
CUBILIN	rs3740165	TT	256	64.2 (20.6)	referent	>0.99	0.41
		TC	16	55.2 (19.9)	-6.95 (-17.52 to 3.63)		
		CC	1*	-	-		
RXRA	rs7861779	GG	198	64.1 (21.2)	referent	0.49	0.18
		GA	64	63.1 (19.3)	-0.86 (-6.89 to 5.17)		
		AA	5	63.9 (10.7)	-6.48 (-25.12 to 12.16)		
	rs9409929	GG	117	66.8 (19.6)	referent	0.37	0.40
		AG	135	61.5 (21.4)	-4.82 (-10.14 to 0.50)		
		AA	22	61.6 (19.0)	-4.49 (-14.45 to 5.46)		
CYP24A1	rs6013897	TT	181	64.6 (20.7)	referent	0.85	0.85
		AT	78	62.7 (20.3)	-1.60 (-7.31 to 4.12)		
		AA	14	59.1 (20.3)	-1.22 (-13.54 to 11.10)		
	rs2762934	GG	195	64.5 (21.2)	referent	0.82	0.50
		AG	74	61.5 (19.0)	-0.92 (-6.66 to 4.81)		
		AA	6	61.8 (23.0)	-1.99 (-18.93 to 14.95)		
	rs2762939	GG	156	64.4 (21.0)	referent	0.31	0.37
		CG	107	62.0 (20.0)	-2.07 (-7.28 to 3.13)		
		CC	12	68.2 (19.6)	6.71 (-6.24 to 19.65)		
	rs2248137	CC	97	63.8 (21.8)	referent	0.71	0.91
		CG	144	63.3 (19.9)	-1.29 (-6.90 to 4.32)		
		GG	33	63.8 (20.7)	-1.60 (-9.93 to 6.74)		
DBP	rs16846876	AA	124	64.3 (21.2)	referent	0.50	0.15
		AT	127	62.8 (20.9)	0.38 (-4.97 to 5.74)		
		TT	24	64.7 (15.2)	3.30 (-6.30 to 12.91)		
	rs7041	CC	65	63.3 (20.8)	referent	0.28	0.61
		AC	148	62.6 (20.1)	-0.36 (-6.45 to 5.73)		
		AA	58	64.7 (21.4)	4.24 (-3.42 to 11.90)		
	rs12512631	TT	114	64.8 (20.3)	referent	0.43	0.72
		CT	131	63.0 (20.6)	-3.20 (-8.74 to 2.33)		
		CC	28	62.0 (22.9)	-3.68 (-12.76 to 5.40)		
	rs4588	GG	130	65.1 (21.1)	referent	0.86	0.040
		GT	124	62.5 (20.2)	-0.98 (-6.29 to 4.33)		
		TT	24	61.0 (18.5)	0.85 (-8.66 to 10.36)		
	rs2070741	TT	238	62.3 (19.9)	referent	0.017	0.25
		TG	31	70.0 (24.5)	7.35 (-0.41 to 15.11)		
		GG	3*	92.4 (11.7)	-		
	rs2298849	AA	182	61.4 (20.5)	referent	0.19	0.97
		AG	88	67.5 (19.9)	7.14 (1.70 to 12.58)		
		GG	7	73.6 (23.6)	10.26 (-5.11 to 25.62)		
CYP27A1	rs17470271	AA	99	58.7 (21.1)	referent	0.11	0.015
		AT	132	67.0 (19.0)	7.23 (1.82 to 12.64)		
		TT	44	64.8 (21.2)	5.87 (-1.38 to 13.13)		
CYP27B1	rs4646536	AA	112	65.1 (21.1)	referent	0.39	0.33
		AG	129	62.2 (20.3)	-3.54 (-9.00 to 1.93)		
		GG	29	63.2 (20.2)	-3.84 (-12.67 to 4.99)		
	rs4646537	TT	254	63.0 (20.6)	referent	0.017	0.75
		GT	21	71.3 (20.2)	11.89 (2.16 to 21.62)		
		GG	0*	-	-		
CYP2R1	rs10500804	TT	96	65.8 (21.4)	referent	0.81	0.83
		GT	132	61.4 (20.8)	-5.29 (-10.94 to 0.35)		
		GG	48	65.3 (17.7)	-0.87 (-8.12 to 6.38)		
	rs2060793	GG	96	63.6 (20.4)	referent	0.59	0.25

Table 5.12 continued.

Gene	SNP	Genotype	N	Mean FEV_1 (SD)	Multivariable model: beta coefficient (95% CI) [1]	P value for trend	P value for genotype* 25(OH)D interaction
		AG	130	63.0 (20.2)	-2.27 (-7.97 to 3.42)		
		AA	49	64.9 (22.4)	2.02 (-5.36 to 9.40)		
	rs10766197	GG	80	66.8 (20.9)	referent	0.62	0.98
		AG	132	61.4 (21.2)	-5.40 (-11.39 to 0.59)		
		AA	58	64.7 (17.4)	-1.79 (-8.85 to 5.28)		
LRP2	rs3755166	GG	92	62.4 (19.3)	referent	0.10	0.97
		AG	126	63.1 (20.2)	2.53 (-3.12 to 8.19)		
		AA	57	67.2 (22.7)	5.64 (-1.16 to 12.45)		
DHCR7	rs12785878	TT	171	63.5 (21.4)	referent	0.30	0.35
		GT	89	65.3 (19.0)	1.36 (-4.07 to 6.80)		
		GG	15	56.9 (19.3)	-6.67 (-19.39 to 6.05)		
	rs3829251	GG	211	64.2 (21.0)	referent	0.59	0.90
		AG	54	61.0 (19.3)	-3.70 (-9.91 to 2.51)		
		AA	7	61.8 (24.2)	4.69 (-12.25 to 21.63)		
VDR	rs731236	AA	104	62.8 (20.6)	referent	0.80	0.63
		AG	127	65.1 (20.6)	-0.99 (-8.50 to 6.52)		
		GG	43	63.1 (18.9)	1.77 (-3.87 to 7.42)		
	rs4334089	GG	146	64.2 (21.2)	referent	0.82	0.40
		AG	108	63.0 (20.1)	-3.21 (-8.58 to 2.16)		
		AA	21	64.4 (17.9)	1.11 (-8.68 to 10.90)		
	rs10783219	AA	106	63.9 (19.4)	referent	0.75	0.60
		AT	128	64.2 (21.1)	-0.26 (-5.84 to 5.33)		
		TT	33	60.1 (21.6)	-1.36 (-9.79 to 7.06)		
	rs4516035	TT	92	61.9 (20.2)	referent	0.52	0.91
		CT	143	63.9 (21.5)	0.02 (-5.72 to 5.77)		
		CC	31	66.9 (18.4)	2.80 (-5.81 to 11.42)		
	rs11568820	CC	165	62.9 (21.4)	referent	0.94	0.60
		CT	98	65.2 (20.0)	0.51 (-12.21 to 13.23)		
		TT	11	61.8 (15.2)	1.66 (-3.71 to 7.02)		
	rs7976091	CC	166	63.2 (21.2)	referent	0.80	0.65
		CT	99	64.7 (20.0)	1.02 (-4.32 to 6.36)		
		TT	12	60.9 (14.8)	-1.63 (-14.01 to 10.75)		
	rs2238136	CC	138	62.0 (18.2)	referent	0.66	0.69
		CT	113	66.2 (22.5)	5.21 (-0.16 to 10.57)		
		TT	19	63.1 (24.6)	2.29 (-8.02 to 12.60)		
	rs1544410	CC	100	62.6 (20.7)	referent	0.98	0.71
		CT	129	64.4 (21.2)	1.57 (-4.06 to 7.20)		
		TT	46	63.7 (19.0)	0.09 (-7.28 to 7.47)		
	rs2228570	GG	106	61.8 (21.4)	referent	0.98	0.11
		AG	125	65.2 (20.7)	3.33 (-2.32 to 8.98)		
		AA	41	62.6 (17.6)	-0.10 (-7.78 to 7.58)		
	rs2853559	GG	96	63.8 (22.1)	referent	0.89	0.82
		AG	131	63.2 (20.1)	-2.37 (-8.08 to 3.34)		
		AA	44	65.1 (19.8)	-0.54 (-8.20 to 7.13)		
	rs7970314	AA	156	63.7 (20.8)	referent	0.68	0.45
		AG	105	63.8 (21.2)	0.25 (-5.05 to 5.55)		
		GG	14	60.7 (13.7)	-2.47 (-14.28 to 9.34)		
	rs7975232	AA	73	62.6 (18.7)	referent	0.39	0.78
		AC	141	64.1 (21.6)	3.45 (-2.78 to 9.69)		
		CC	55	63.7 (21.0)	3.24 (-4.23 to 10.71)		

[1] *Adjusted for potential determinants of FEV_1: Sex, age, ethnicity, body mass index, socio-economic position, smoking status, alcohol consumption, influenza vaccination, pneumococcal vaccination, and baseline vitamin D status. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for trend or interaction between baseline vitamin D status and genotype remain significant.*

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor A, VDR: Vitamin D receptor, CUBN: Cubilin.

Table 5.13. Genetic determinants of forced vital capacity (FVC).

Gene	SNP	Genotype	N	Mean FVC (SD)	Multivariable model: beta coefficient (95% CI) [1]	P value for trend	P value for genotype* 25(OH)D Interaction
CYP3A4	rs2740574	AA	247	97.5 (19.3)	referent	0.48	0.29
		AG	26	91.7 (16.1)	-7.10 (-14.85 to 0.65)		
		GG	1*	-	-		
CUBILIN	rs3740165	TT	256	97.5 (18.9)	referent	0.88	0.27
		TC	16	85.5 (19.5)	-9.20 (-18.54 to 0.15)		
		CC	1*	-	-		
RXRA	rs7861779	GG	198	97.8 (19.4)	referent	0.33	0.11
		GA	64	94.2 (18.2)	-3.54 (-8.89 to 1.81)		
		AA	5	90.9 (13.8)	-8.18 (-24.71 to 8.35)		
	rs9409929	GG	117	97.7 (19.0)	referent	0.17	0.018
		AG	135	95.3 (19.7)	0.01 (-4.74 to 4.76)		
		AA	22	104.3 (15.4)	6.25 (-2.63 to 15.14)		
CYP24A1	rs6013897	TT	181	98.0 (19.5)	referent	0.59	0.74
		AT	78	96.3 (18.4)	-1.18 (-6.28 to 3.91)		
		AA	14	88.0 (16.9)	-2.97 (-13.96 to 8.02)		
	rs2762934	GG	195	97.7 (19.5)	referent	0.67	0.19
		AG	74	93.7 (18.0)	-1.93 (-7.05 to 3.18)		
		AA	6	104.5 (17.8)	3.23 (-11.87 to 18.34)		
	rs2762939	GG	156	97.1 (20.1)	referent	0.037	0.97
		CG	107	95.1 (17.3)	-2.24 (-6.84 to 2.37)		
		CC	12	108.0 (19.8)	12.19 (0.73 to 23.65)		
	rs2248137	CC	97	96.4 (20.1)	referent	0.91	0.74
		CG	144	96.9 (18.7)	-0.55 (-5.57 to 4.46)		
		GG	33	97.0 (18.4)	0.42 (-7.04 to 7.87)		
DBP	rs16846876	AA	124	99.08 (20.0)	referent	0.67	0.92
		AT	127	95.3 (18.7)	-2.14 (-6.91 to 2.63)		
		TT	24	94.2 (15.9)	-1.85 (-10.40 to 6.70)		
	rs7041	CC	65	98.6 (17.5)	referent	0.66	0.51
		AC	148	96.9 (20.0)	-0.15 (-5.65 to 5.36)		
		AA	58	94.1 (19.4)	-1.55 (-8.48 to 5.37)		
	rs12512631	TT	114	97.6 (18.9)	referent	0.56	0.28
		CT	131	96.8 (19.0)	-1.46 (-6.42 to 3.50)		
		CC	28	94.9 (22.2)	-2.41 (-10.54 to 5.73)		
	rs4588	GG	130	100.1 (19.5)	referent	0.11	0.62
		GT	124	95.0 (19.0)	-3.73 (-8.45 to 0.99)		
		TT	24	90.4 (15.8)	-6.79 (-15.25 to 1.66)		
	rs2070741	TT	238	95.7 (18.7)	referent	0.26	0.44
		TG	31	102.7 (20.1)	5.01 (-1.88 to 11.90)		
		GG	3*	105.9 (15.3)	-		
	rs2298849	AA	182	95.0 (19.7)	referent	0.48	0.65
		AG	88	100.4 (17.2)	6.53 (1.68 to 11.39)		
		GG	7	102.8 (20.7)	4.89 (-8.83 to 18.61)		
CYP27A1	rs17470271	AA	99	95.0 (18.8)	referent	0.67	0.16
		AT	132	99.0 (18.3)	2.18 (-2.69 to 7.06)		
		TT	44	95.5 (20.8)	-1.41 (-7.94 to 5.13)		
CYP27B1	rs4646536	AA	112	95.4 (19.9)	referent	0.41	0.15
		AG	129	98.4 (19.1)	1.17 (-3.73 to 6.07)		
		GG	29	95.7 (18.5)	-3.33 (-11.2 to 4.58)		
	rs4646537	TT	254	96.6 (19.1)	referent	0.11	0.71
		GT	21	99.3 (20.3)	7.02 (-1.69 to 15.73)		
		GG	0*	-	-		
CYP2R1	rs10500804	TT	96	96.7 (19.7)	referent	0.87	0.30
		GT	132	97.1 (19.2)	0.97 (-4.13 to 6.06)		
		GG	48	97.2 (18.8)	0.54 (-6.00 to 7.08)		
	rs2060793	GG	96	96.8 (19.0)	referent	0.96	0.73

Table 5.13 continued.

Gene	SNP	Genotype	N	Mean FVC (SD)	Multivariable model: beta coefficient (95% CI) [1]	P value for trend	P value for genotype* 25(OH)D Interaction
		AG	130	97.2 (19.2)	0.44 (-4.65 to 5.54)		
		AA	49	96.5 (20.1)	-0.18 (-6.79 to 6.43)		
	rs10766197	GG	80	97.1 (19.6)	referent	0.53	0.20
		AG	132	96.6 (18.6)	0.84 (-4.55 to 6.23)		
		AA	58	98.5 (19.5)	2.04 (-4.32 to 8.40)		
LRP2	rs3755166	GG	92	96.8 (19.8)	referent	0.42	0.81
		AG	126	96.5 (19.1)	1.94 (-3.12 to 6.99)		
		AA	57	98.4 (17.6)	2.47 (-3.61 to 8.55)		
DHCR7	rs12785878	TT	171	96.8 (19.4)	referent	0.48	0.45
		GT	89	97.6 (17.8)	1.70 (-3.09 to 6.49)		
		GG	15	91.2 (19.2)	4.03 (-7.19 to 15.24)		
	rs3829251	GG	211	97.2 (19.1)	referent	0.14	0.97
		AG	54	94.1 (17.1)	-2.96 (-8.41 to 2.48)		
		AA	7	95.4 (25.4)	11.04 (-3.80 to 25.89)		
VDR	rs731236	AA	104	92.9 (19.0)	referent	0.16	0.14
		AG	127	100.1 (18.3)	6.61 (1.65 to 11.58)		
		GG	43	98.9 (19.1)	4.76 (-1.85 to 11.37)		
	rs4334089	GG	146	98.3 (19.2)	referent	0.68	0.55
		AG	108	94.9 (18.8)	-4.03 (-8.82 to 0.76)		
		AA	21	99.6 (19.3)	1.83 (-6.90 to 10.56)		
	rs10783219	AA	106	96.6 (19.1)	referent	0.68	0.32
		AT	128	97.7 (19.0)	0.34 (-4.63 to 5.32)		
		TT	33	94.1 (19.0)	-1.60 (-9.10 to 5.91)		
	rs4516035	TT	92	95.5 (19.5)	referent	0.58	0.71
		CT	143	97.9 (19.2)	-0.02 (-5.18 to 5.14)		
		CC	31	96.2 (18.3)	-2.18 (-9.92 to 5.55)		
	rs11568820	CC	165	96.6 (19.8)	referent	0.11	0.46
		CT	98	96.7 (17.1)	0.58 (-4.17 to 5.33)		
		TT	11	104.6 (26.0)	9.06 (-2.19 to 20.32)		
	rs7976091	CC	166	96.8 (19.7)	referent	0.29	0.51
		CT	99	96.4 (17.1)	0.18 (-4.57 to 4.94)		
		TT	12	101.6 (26.9)	5.96 (-5.07 to 16.98)		
	rs2238136	CC	138	95.9 (19.1)	referent	0.74	0.32
		CT	113	98.2 (18.9)	2.59 (-2.26 to 7.44)		
		TT	19	94.4 (21.6)	-1.58 (-10.90 to 7.74)		
	rs1544410	CC	100	93.2 (18.7)	referent	0.25	0.084
		CT	129	99.4 (19.3)	5.86 (0.88 to 10.83)		
		TT	46	97.2 (18.5)	3.80 (-2.72 to 10.31)		
	rs2228570	GG	106	95.0 (20.2)	referent	0.38	0.59
		AG	125	99.0 (18.7)	3.34 (-1.68 to 8.37)		
		AA	41	94.3 (17.4)	-3.03 (-9.86 to 3.80)		
	rs2853559	GG	96	95.5 (19.9)	referent	0.93	0.76
		AG	131	97.4 (18.7)	-0.55 (-5.63 to 4.53)		
		AA	44	97.6 (19.7)	-0.29 (-7.12 to 6.54)		
	rs7970314	AA	156	97.2 (19.6)	referent	0.30	0.42
		AG	105	95.7 (17.6)	-0.65 (-5.37 to 4.07)		
		GG	14	101.1 (25.6)	5.53 (-4.97 to 16.04)		
	rs7975232	AA	73	98.6 (18.6)	referent	0.34	0.34
		AC	141	97.3 (20.1)	-0.91 (-6.46 to 4.64)		
		CC	55	92.7 (17.9)	-3.21 (-9.86 to 3.44)		

[1] *Adjusted for potential determinants of FVC: Sex, age, ethnicity, body mass index, socio-economic position, smoking status, alcohol consumption, influenza vaccination, pneumococcal vaccination, and baseline vitamin D status. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for trend or interaction between baseline vitamin D status and genotype remain significant.*

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, SNP: Single nucleotide polymorphism, SD: Standard deviation, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor, CUBN: Cubilin.

Table 5.14. Genetic determinants of FEV_1:FVC.

Gene	SNP	Genotype	N	Mean FEV_1:FVC (SD)	Multivariable model: beta coefficient (95% CI) [1]	P value for trend	P value for genotype* 25(OH)D interaction
CYP3A4	rs2740574	AA	247	0.52 (0.13)	referent	0.95	0.68
		AG	26	0.53 (0.10)	-0.01 (-0.06 to 0.04)		
		GG	1*	-	-		
CUBILIN	rs3740165	TT	256	0.52 (0.13)	referent	0.92	0.92
		TC	16	0.50 (0.13)	-0.01 (-0.07 to 0.06)		
		CC	1*	-	-		
RXRA	rs7861779	GG	198	0.52 (0.13)	referent	0.99	0.41
		GA	64	0.53 (0.12)	0.02 (-0.02 to 0.05)		
		AA	5	0.58 (0.06)	0.00 (-0.11 to 0.11)		
	rs9409929	GG	117	0.55 (0.11)	referent	0.027	0.84
		AG	135	0.51 (0.13)	-0.04 (-0.07 to -0.01)		
		AA	22	0.47 (0.13)	-0.07 (-0.13 to -0.01)		
CYP24A1	rs6013897	TT	181	0.52 (0.13)	referent	0.92	0.83
		AT	78	0.51 (0.13)	-0.01 (-0.04 to 0.03)		
		AA	14	0.52 (0.12)	0.04 (-0.07 to 0.08)		
	rs2762934	GG	195	0.52 (0.13)	referent	0.36	0.87
		AG	74	0.52 (0.12)	0.01 (-0.03 to 0.04)		
		AA	6	0.46 (0.11)	-0.05 (-0.15 to 0.05)		
	rs2762939	GG	156	0.53 (0.13)	referent	0.79	0.40
		CG	107	0.51 (0.13)	-0.00 (-0.04 to 0.03)		
		CC	12	0.50 (0.10)	-0.01 (-0.09 to 0.07)		
	rs2248137	CC	97	0.52 (0.13)	referent	0.63	0.73
		CG	144	0.52 (0.13)	-0.00 (-0.04 to 0.03)		
		GG	33	0.52 (0.12)	-0.01 (-0.06 to 0.04)		
DBP	rs16846876	AA	124	0.52 (0.13)	referent	0.15	0.076
		AT	127	0.52 (0.13)	0.02 (-0.02 to 0.05)		
		TT	24	0.54 (0.10)	0.04 (-0.02 to 0.10)		
	rs7041	CC	65	0.51 (0.13)	referent	0.094	0.74
		AC	148	0.51 (0.13)	-0.00 (-0.04 to 0.04)		
		AA	58	0.54 (0.13)	0.04 (-0.01 to 0.09)		
	rs12512631	TT	114	0.53 (0.13)	referent	0.47	0.19
		CT	131	0.51 (0.13)	-0.02 (-0.05 to 0.01)		
		CC	28	0.53 (0.13)	-0.02 (-0.07 to 0.03)		
	rs4588	GG	130	0.52 (0.13)	referent	0.10	0.014
		GT	124	0.52 (0.13)	0.01 (-0.02 to 0.04)		
		TT	24	0.53 (0.13)	0.05 (-0.01 to 0.11)		
	rs2070741	TT	238	0.52 (0.12)	referent	0.051	0.33
		TG	31	0.54 (0.14)	0.03 (-0.02 to 0.07)		
		GG	3*	0.68 (0.05)	-		
	rs2298849	AA	182	0.51 (0.13)	referent	0.28	0.92
		AG	88	0.53 (0.12)	0.02 (-0.01 to 0.06)		
		GG	7	0.58 (0.12)	0.05 (-0.04 to 0.15)		
CYP27A1	rs17470271	AA	99	0.49 (0.14)	referent	0.024	0.020
		AT	132	0.54 (0.11)	0.05 (0.01 to 0.08)		
		TT	44	0.54 (0.13)	0.05 (0.01 to 0.09)		
CYP27B1	rs4646536	AA	112	0.54 (0.13)	referent	0.91	0.86
		AG	129	0.50 (0.12)	-0.03 (-0.07 to -0.00)		
		GG	29	0.54 (0.13)	-0.00 (-0.06 to 0.05)		
	rs4646537	TT	254	0.52 (0.13)	referent	0.031	0.91
		GT	21	0.57 (0.12)	0.07 (0.01 to 0.12)		
		GG	0*	-	-		
CYP2R1	rs10500804	TT	96	0.54 (0.12)	referent	0.85	0.29
		GT	132	0.50 (0.13)	-0.05 (-0.08 to -0.01)		
		GG	48	0.53 (0.12)	-0.00 (-0.05 to 0.04)		
	rs2060793	GG	96	0.52 (0.13)	referent	0.49	0.23

Table 5.14 continued.

Gene	SNP	Genotype	N	Mean FEV_1:FVC (SD)	Multivariable model: beta coefficient (95% CI) [1]	P value for trend	P value for genotype* 25(OH)D interaction
		AG	130	0.51 (0.13)	-0.02 (-0.06 to 0.01)		
		AA	49	0.53 (0.13)	0.02 (-0.03 to 0.06)		
	rs10766197	GG	80	0.55 (0.11)	referent	0.34	0.45
		AG	132	0.50 (0.13)	-0.05 (-0.09 to -0.02)		
		AA	58	0.53 (0.12)	-0.02 (-0.06 to 0.02)		
LRP2	rs3755166	GG	92	0.52 (0.13)	referent	0.26	0.96
		AG	126	0.52 (0.13)	0.01 (0.03 to 0.04)		
		AA	57	0.54 (0.13)	0.02 (-0.02 to 0.07)		
DHCR7	rs12785878	TT	171	0.52 (0.13)	referent	0.12	0.055
		GT	89	0.53 (0.13)	0.01 (-0.02 to 0.04)		
		GG	15	0.50 (0.14)	-0.06 (-0.14 to 0.02)		
	rs3829251	GG	211	0.52 (0.13)	referent	0.82	0.92
		AG	54	0.52 (0.13)	-0.01 (-0.04 to 0.03)		
		AA	7	0.51 (0.13)	-0.01 (-0.11 to 0.09)		
VDR	rs731236	AA	104	0.53 (0.12)	referent	0.28	0.19
		AG	127	0.52 (0.13)	-0.02 (-0.05 to 0.02)		
		GG	43	0.51 (0.14)	-0.03 (-0.07 to 0.02)		
	rs4334089	GG	146	0.52 (0.13)	referent	0.81	0.39
		AG	108	0.53 (0.13)	-0.01 (-0.04 to 0.03)		
		AA	21	0.51 (0.12)	0.01 (-0.05 to 0.07)		
	rs10783219	AA	106	0.52 (0.12)	referent	0.77	0.94
		AT	128	0.52 (0.13)	-0.00 (-0.04 to 0.03)		
		TT	33	0.50 (0.13)	-0.01 (-0.06 to 0.04)		
	rs4516035	TT	92	0.51 (0.12)	referent	0.14	0.80
		CT	143	0.52 (0.13)	0.00 (-0.03 to 0.04)		
		CC	31	0.55 (0.12)	0.04 (-0.01 to 0.09)		
	rs11568820	CC	165	0.52 (0.13)	referent	0.45	0.74
		CT	98	0.53 (0.13)	0.01 (-0.03 to 0.04)		
		TT	11	0.47 (0.09)	-0.03 (-0.11 to 0.05)		
	rs7976091	CC	166	0.52 (0.13)	referent	0.40	0.78
		CT	99	0.53 (0.13)	0.00 (-0.03 to 0.04)		
		TT	12	0.48 (0.09)	-0.03 (-0.11 to 0.04)		
	rs2238136	CC	138	0.51 (0.12)	referent	0.59	0.99
		CT	113	0.53 (0.13)	0.02 (-0.01 to 0.06)		
		TT	19	0.53 (0.14)	0.02 (-0.04 to 0.08)		
	rs1544410	CC	100	0.53 (0.12)	referent	0.58	0.17
		CT	129	0.51 (0.13)	-0.02 (-0.05 to 0.02)		
		TT	46	0.52 (0.14)	-0.01 (-0.06 to 0.03)		
	rs2228570	GG	106	0.51 (0.13)	referent	0.31	0.13
		AG	125	0.52 (0.13)	0.01 (-0.03 to 0.04)		
		AA	41	0.53 (0.12)	0.02 (-0.02 to 0.07)		
	rs2853559	GG	96	0.52 (0.13)	referent	0.97	0.91
		AG	131	0.52 (0.12)	-0.01 (-0.05 to 0.02)		
		AA	44	0.53 (0.14)	0.00 (-0.05 to 0.05)		
	rs7970314	AA	156	0.52 (0.13)	referent	0.32	0.55
		AG	105	0.52 (0.13)	0.00 (-0.03 to 0.03)		
		GG	14	0.49 (0.08)	-0.04 (-0.11 to 0.04)		
	rs7975232	AA	73	0.51 (0.14)	referent	0.094	0.48
		AC	141	0.52 (0.12)	0.03 (-0.01 to 0.06)		
		CC	55	0.54 (0.12)	0.04 (-0.01 to 0.08)		

[1] *Adjusted for potential determinants of FEV_1:FVC: Sex, age, ethnicity, body mass index, socio-economic position, smoking status, alcohol consumption, influenza vaccination, pneumococcal vaccination, and baseline vitamin D status. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for trend or interaction between baseline vitamin D status and genotype remain significant.*

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, SNP: Single nucleotide polymorphism, SD: Standard deviation, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor, CUBN: Cubilin.

Table 5.15. Genetic determinants of quadriceps strength (QS).

Gene	SNP	Genotype	N [1]	Mean QS, kg (SD)	Multivariable model: beta coefficient (95% CI) [2]	P value for trend	P value for genotype* 25(OH) D interaction
CYP3A4	rs2740574	AA	119	32.8 (11.9)	referent	0.80	0.17
		AG	14	30.3 (15.7)	-0.78 (-6.75 to 5.20)		
		GG	0*	-	-		
CUBILIN	rs3740165	TT	123	32.3 (12.6)	referent	0.087	0.98
		TC	9	33.5 (6.4)	1.69 (-5.79 to 9.18)		
		CC	1*	-	-		
RXRA	rs7861779	GG	92	32.4 (11.6)	referent	0.67	0.53
		GA	33	31.7 (13.3)	-1.17 (-5.28 to 2.94)		
		AA	3*	40.6 (8.3)	-		
	rs9409929	GG	58	30.6 (12.0)	referent	0.42	0.64
		AG	61	33.9 (12.5)	2.27 (-1.77 to 6.31)		
		AA	13	35.3 (12.4)	2.89 (-4.12 to 9.91)		
CYP24A1	rs6013897	TT	87	33.1 (12.0)	referent	0.78	0.90
		AT	40	30.7 (12.8)	-2.41 (-6.57 to 1.75)		
		AA	6	35.9 (13.5)	1.36 (-8.25 to 10.95)		
	rs2762934	GG	93	32.6 (11.7)	referent	0.82	0.11
		AG	38	32.6 (13.9)	0.25 (-3.82 to 4.31)		
		AA	3	31.1 (12.0)	-1.43 (-13.95 to 11.09)		
	rs2762939	GG	68	32.9 (12.8)	referent	0.62	0.025
		CG	54	32.1 (12.4)	-0.16 (-4.02 to 3.71)		
		CC	10	34.5 (8.2)	1.85 (-5.44 to 9.14)		
	rs2248137	CC	47	33.8 (11.8)	referent	0.99	0.98
		CG	69	32.1 (12.7)	-0.01 (-4.34 to 4.31)		
		GG	17	31.9 (12.2)	0.04 (-6.30 to 6.38)		
DBP	rs16846876	AA	60	34.6 (11.3)	referent	0.56	0.12
		AT	63	29.8 (10.7)	-2.80 (-6.78 to 1.19)		
		TT	10	34.9 (20.4)	2.10 (-5.04 to 9.24)		
	rs7041	CC	35	30.9 (10.8)	referent	0.094	0.73
		AC	72	32.6 (13.3)	4.47 (-0.03 to 8.91)		
		AA	23	34.3 (11.5)	5.05 (-0.87 to 10.98)		
	rs12512631	TT	51	31.7 (12.9)	referent	0.43	0.73
		CT	70	33.1 (11.9)	-0.14 (-4.19 to 3.92)		
		CC	13	33.0 (12.7)	-2.69 (-9.49 to 4.11)		
	rs4588	GG	63	32.9 (11.6)	referent	0.38	0.63
		GT	59	32.5 (12.7)	-0.03 (-3.96 to 3.89)		
		TT	12	31.2 (14.4)	3.13 (-3.93 to 10.20)		
	rs2070741	TT	120	32.8 (12.6)	referent	0.82	0.59
		TG	12	28.9 (8.9)	0.48 (-5.79 to 6.75)		
		GG	2*	39.8 (6.6)	-		
	rs2298849	AA	91	33.9 (11.8)	referent	0.98	0.34
		AG	39	30.7 (13.0)	-3.55 (-7.81 to 0.72)		
		GG	4*	20.4 (4.7)	-		
CYP27A1	rs17470271	AA	46	33.8 (11.8)	referent	0.51	0.69
		AT	60	31.9 (13.3)	-0.26 (-4.50 to 3.97)		
		TT	27	32.3 (10.9)	-1.74 (-6.95 to 3.47)		
CYP27B1	rs4646536	AA	57	33.2 (12.6)	referent	0.26	0.62
		AG	58	31.6 (12.5)	-0.19 (-4.26 to 3.88)		
		GG	16	35.1 (11.2)	3.65 (-2.69 to 10.00)		
	rs4646537	TT	122	32.6 (12.5)	referent	0.50	0.56
		GT	12	32.5 (10.1)	-2.32 (-9.06 to 4.43)		
		GG	0*	-	-		
CYP2R1	rs10500804	TT	44	30.4 (13.5)	referent	0.58	0.55
		GT	61	33.9 (13.2)	2.07 (-2.29 to 6.42)		
		GG	28	32.8 (7.2)	1.49 (-3.81 to 6.79)		
	rs2060793	GG	50	33.2 (10.9)	referent	0.11	0.12

Table 5.15 continued.

Gene	SNP	Genotype	N [1]	Mean QS, kg (SD)	Multivariable model: beta coefficient (95% CI) [2]	P value for trend	P value for genotype* 25(OH) D interaction
		AG	63	33.9 (13.5)	0.90 (-3.29 to 5.09)		
		AA	21	27.0 (10.2)	-4.53 (-10.05 to 0.99)		
	rs10766197	GG	37	30.8 (14.3)	referent	0.40	0.17
		AG	63	33.2 (13.0)	1.16 (-3.43 to 5.75)		
		AA	32	33.1 (7.9)	2.28 (-3.02 to 7.58)		
LRP2	rs3755166	GG	45	34.6 (13.4)	referent	0.067	0.77
		AG	59	31.6 (11.6)	-2.72 (-7.01 to 1.56)		
		AA	30	31.4 (11.7)	-4.59 (-9.50 to 0.33)		
DHCR7	rs12785878	TT	78	31.3 (12.1)	referent	0.34	0.19
		GT	44	32.9 (13.2)	0.71 (-3.30 to 4.71)		
		GG	11	39.9 (7.4)	3.73 (-3.92 to 11.38)		
	rs3829251	GG	100	32.1 (12.1)	referent	0.13	0.71
		AG	28	32.6 (13.2)	1.46 (-2.97 to 5.89)		
		AA	5	40.1 (10.3)	7.36 (-2.25 to 16.97)		
VDR	rs731236	AA	50	30.6 (12.6)	referent	0.84	0.41
		AG	65	34.0 (11.9)	2.32 (-1.87 to 6.51)		
		GG	19	32.7 (12.7)	-0.60 (-6.48 to 5.28)		
	rs4334089	GG	70	31.4 (13.3)	referent	0.53	0.48
		AG	51	33.7 (11.5)	1.00 (-2.98 to 4.99)		
		AA	13	34.4 (9.1)	-2.05 (-8.58 to 4.48)		
	rs10783219	AA	54	33.7 (10.7)	referent	0.28	0.45
		AT	64	32.3 (13.4)	1.20 (-2.83 to 5.23)		
		TT	15	29.9 (12.9)	-3.36 (-9.45 to 2.74)		
	rs4516035	TT	46	32.1 (11.2)	referent	0.49	0.35
		CT	73	32.0 (13.2)	1.84 (-2.29 to 5.98)		
		CC	13	35.8 (11.3)	2.45 (-4.52 to 9.42)		
	rs11568820	CC	80	32.0 (13.4)	referent	0.74	0.68
		CT	45	33.6 (10.9)	0.41 (-3.65 to 4.47)		
		TT	9	32.5 (7.8)	-1.22 (-8.56 to 6.13)		
	rs7976091	CC	79	32.1 (13.4)	referent	0.70	0.78
		CT	46	33.4 (10.9)	-0.09 (-4.14 to 3.96)		
		TT	9	32.5 (7.8)	-1.42 (-8.79 to 5.95)		
	rs2238136	CC	66	33.7 (10.5)	referent	0.37	0.73
		CT	59	31.5 (14.0)	0.23 (-3.83 to 4.29)		
		TT	8	32.6 (12.7)	-4.16 (-13.22 to 4.91)		
	rs1544410	CC	48	30.5 (12.6)	referent	0.79	0.40
		CT	66	34.0 (11.9)	2.42 (-1.70 to 6.55)		
		TT	20	32.8 (12.4)	-0.79 (-6.49 to 4.92)		
	rs2228570	GG	51	32.9 (12.0)	referent	0.99	0.82
		AG	64	32.2 (12.4)	0.42 (-3.62 to 4.46)		
		AA	18	32.6 (13.2)	0.03 (-5.92 to 5.98)		
	rs2853559	GG	49	33.6 (10.9)	referent	0.87	0.10
		AG	67	32.2 (13.2)	1.72 (-2.40 to 5.85)		
		AA	17	31.8 (12.8)	-0.50 (-6.56 to 5.55)		
	rs7970314	AA	71	32.3 (13.5)	referent	0.54	0.88
		AG	52	32.8 (11.4)	-0.25 (-4.16 to 3.67)		
		GG	10	32.4 (7.3)	-2.21 (-9.43 to 5.00)		
	rs7975232	AA	33	30.7 (11.6)	referent	0.49	0.93
		AC	72	33.2 (12.6)	4.55 (0.01 to 9.09)		
		CC	25	31.8 (12.7)	1.97 (-3.60 to 7.53)		

[1] *Quadriceps strength measured in a subset of n=134.* ***[2]*** *Adjusted for potential determinants of quadriceps strength: Sex, age, body mass index, socio-economic position, smoking status, alcohol consumption, and baseline vitamin D status. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for trend or interaction between baseline vitamin D status and genotype remain significant.*

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, SNP: Single nucleotide polymorphism, SD: Standard deviation, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor, CUBN: Cubilin.

6.3. Discussion.

To my knowledge this study represents the most comprehensive cross-sectional analysis of environmental and genetic determinants of serum 25(OH)D concentration, and clinical correlates of disease phenotype in a UK cohort of COPD patients. It is also the first to explore the influence of vitamin D deficiency on induced sputum biomarkers of disease severity in this patient group, and SNP in genes other than *DBP* and *VDR.*

Vitamin D deficiency (defined by a serum 25[OH]D concentration <50 nmol/L) was present in the majority of participants (61.5%). Serum 25(OH)D concentration independently associated with BMI, SEP, vitamin D supplement consumption, quarter of sampling, and holidaying abroad, but not with any of the 15 investigated SNP which have been found to in healthy populations. Vitamin D showed an association with reduced lung function, as measured by % predicted FEV_1 and % predicted FVC, but did not associate with the remaining investigated clinical correlates of COPD severity or control, namely: FEV_1:FVC ratio, ICS requirement, health-related quality of life score, quadriceps strength, or % eosinophils or neutrophils in induced sputum.

The findings I present on environmental determinants of vitamin D status are in agreement with previous findings in the healthy, UK population (298). With respect to our findings on genetic determinants of vitamin D status, before correcting for multiple comparison testing, two of our fifteen SNP (all of which have been shown to predict vitamin D status in healthy populations, summarised in (335)) independently associate with serum 25(OH)D concentration in our COPD cohort. TT genotype for one of these SNP – rs7041 in *DBP* – has previously been reported to associate with a 25% decrease in serum 25(OH)D concentration in COPD patients, compared to GG genotype (323); I found a 23.1% decrease in adjusted mean concentration for the same comparison, which raises the question of possible type II error resulting from multiple comparisons correction. With

respect to the null results of our analysis of potential genetic determinants of COPD phenotype: very few studies have investigated a potential association in this population, but one case-control study has reported an association between two DBP SNP (rs1155563 and rs17467825, that are tagged by a SNP in our panel [rs4588]) and % predicted FEV1, and FEV1/FVC. This team also replicated their finding in a second large cohort (336). Interestingly, I observed an interaction between rs4588 genotype and baseline 25(OH)D concentration on the same outcomes of % predicted FEV1, and FEV1/FVC (P for interaction = 0.040 and 0.014, respectively), however these findings did not survive correction for multiple comparison testing. This may have been due to our relative lack of power to detect genotypic subgroup effects, or it may reflect type II error due to strict statistical correction for multiple comparisons, or it may be that vitamin D does not influence COPD phenotype and previous findings have arisen due to residual confounding.

The associations I observed between vitamin D status and spiromteric indices, namely % predicted FEV_1 and % predicted FVC, are consistent with previous reports (323, 324, 326-328). Further research is required to establish whether vitamin D deficiency is a cause or effect of this particular marker of COPD severity, or whether the association that I and others have observed is due to residual confounding. The lack of association I report between vitamin D status and % eosinophils and neutrophils in sputum is a novel finding. Limited intervention data report a reduction in asthmatic airway eosinophilia with vitamin D supplementation (337), thus one might expect to see an association in COPD patients – both being chronic respiratory diseases with certain immunopathological parallels. That I did not observe an association may be due to the relatively small subset (n=44) of participants I tested, or it may be that these cellular mediators of inflammation are not modulated by vitamin D. Our findings of no association between vitamin D status and FEV_1:FVC, or quadriceps strength are supported by previous observational studies (324, 326, 330), whilst the lack of association between vitamin D status and ICS requirement I report is in contrast to a previous case-control study (328) conducted in Norway which reported a 5 nmol/L lower mean

serum 25(OH)D concentration in COPD cases using ICS vs. those who were not (P=0.05). However the analysis by Persson et al (328) was a univariate analysis, so factors which may confound the relationship between vitamin D status and ICS requirement were not controlled for.

My analysis validated a number of other expected predictors of COPD phenotype, but also identified a few unexpected associations. Compared to the youngest COPD patients (1st quartile – 44 to 59 yrs) the oldest (4th quartile - 70 to 92 years) showed a slightly smaller drop in quadriceps strength than those in the 3rd quartile for age (65 to 70 years). This suggests the process of diminishing skeletal muscle in COPD may reach a plateau around 70 years of age. Alternatively, it may be that better disease management occurs in patients of more advanced age as a result of a greater impetus to participate in pulmonary rehabilitation classes. Another unexpected finding was the observation of better SGRQ score with increasing age. As there was an overall trend for worsening COPD control and severity with advancing age for most of our clinical correlates, one might expect to see poorer mean SGRQ scores in quartiles 2-4 of age, but the fact these data arise from self-report is most likely key – elderly participants may adopt a more modest attitude when describing the severity of their own illness, or lower expectations of QoL.

6.3.1. Study strengths.

My study has several strengths. I characterised participants in a considerable level of detail which allowed me to investigate a wide range of potential genetic and environmental determinants of vitamin D status; control for a wide range of potential factors which may confound the relationship between vitamin D status and COPD severity and control; and conduct a comprehensive analysis of potential determinants of COPD phenotype. Spirometry was performed using international guidelines and serum 25(OH)D concentrations were measured with the gold standard assay (LC-

MS/MS) in a laboratory that participated in the international vitamin D external quality assurance scheme (www.deqas.org/). The study population included COPD patients with a range of disease severity, whom were recruited and followed up in both community and hospital settings and across all four seasons – all of which are factors that enhance the generalisibility of my results. Finally, to our knowledge, this was the first study of COPD patients to investigate a cross-sectional relationship between vitamin D status and sputum biomarkers.

6.3.2. Study limitations.

This study is not without its limitations: due to the prevalence of vitamin D deficiency in the cohort only a little over 10% of participants had a serum 25(OH)D concentration >75 nmol/L, therefore I may have been underpowered to detect the influence of optimal vitamin D status on COPD phenotype. Type 2 error as a result of limited power may also have been an issue in our analysis of quadriceps strength, which was conducted in a subset of 134 patients, and sputum markers of COPD phenotype, which was conducted in a subset of 44 patients.

5.7. Conclusions.

In conclusion, my findings highlight a high prevalence of vitamin D deficiency in a UK population of COPD patients. I found independent associations between low serum 25(OH)D concentrations and greater disease severity as indicated by reduced % predicted FEV1 and FVC; higher BMI, lower SEP, lack of vitamin D supplement consumption, sampling during winter/spring, and lack of holidaying abroad. I found no association between genotype and clinical correlates of COPD phenotype, either directly, or in interaction with vitamin D status which does not answer the question of possible reverse causation between vitamin D deficiency and severity of COPD phenotype. These findings will help clinicians to identify COPD patients who are at particular risk of vitamin D deficiency, and

support the need to correct levels with an intervention that has now been shown to reduce risk of COPD exacerbations in deficient subgroups of two clinical trials (210, 272).

7. Genetic variants in the vitamin D pathway and susceptibility to acute respiratory infections in patients with asthma.

This chapter presents the findings of an investigation into the relationship between single nucleotide polymorphisms in the vitamin D pathway and the risk of URI or disease exacerbation in patients with ICS-treated asthma, who participated in the ViDiAs trial (described in Methods, section 2.2.1). This will comprise analyses of SNP genotype association with URI / exacerbation directly (main effects), and with modified effect of vitamin D supplementation to prevent URI / exacerbation (effect modification).

7.1. Introduction.

The potential role for vitamin D supplementation as a preventive treatment for acute respiratory infection (ARI) may be of particular significance to asthma sufferers in whom ARI commonly precipitate acute symptom exacerbations, for which there are limited interventions to prevent (39). Numerous observational studies have reported an association between vitamin D deficiency (defined by a serum 25-hydroxyvitamin D concentration <50 nmol/L) and increased risk of ARI (231) which is most significant in patients with asthma (338), and between vitamin D deficiency and markers of asthma severity such as poor lung function, lower asthma control test results, and increased requirement for inhaled corticosteroids (ICS) (245, 291-294). Results from clinical trials of vitamin D supplementation in the prevention of ARI and/or disease exacerbation in asthma cohorts are conflicting however: two trials conducted on children report significant protective effects (280, 299) whilst two conducted on adults report a null outcome, one of which is the ViDiAs trial (209, 300). The lack of effect in the latter two trials may be due to power issues, low prevalence of vitamin D deficiency at baseline, or sub-optimal dosing regimens. Another factor which could explain this heterogeneity is the potential effect that variation in vitamin D pathway genes might exert on risk of

ARI, either directly or by modifying the effects of vitamin D supplementation. I recently reviewed the literature surrounding variation in vitamin D genes and found reports of 55 single nucleotide polymorphisms (SNP) in genes encoding proteins involved in vitamin D metabolism, transportation, and signalling, that associate with circulating concentrations of 25-hydroxyvitamin D (25[OH]D), 1,25-dihydroxyvitamin D ($1,25[OH]_2D$), and/or non-skeletal health outcomes (335). Variation in the vitamin D binding protein (DBP) gene, a cytochrome P450 enzyme (CYP2R1) gene, and the 7-dehydrocholesterol reductase (DHCR7) gene most commonly associate with vitamin D metabolite concentrations, whilst variation in the vitamin D receptor (VDR) gene associates with the majority of reports on non-skeletal health outcomes, of which infectious and autoimmune diseases were the most common groups (335). The *VDR* mutation, *FokI* (rs10735810/rs2228570) has been found to associate with risk of respiratory syncytial virus (RSV) infection and lower respiratory infection (LRI) in cohorts from the Netherlands (339), South Africa (340), and Canada (202). *FokI* has also been found to associate with markers of asthma phenotype: IgE concentration, spirometric lung function indices (forced expiratory volume in 1 second to forced vital capacity ratio [FEV_1:FVC]), Th2 cytokine concentration, and overall risk of asthma (130, 195). The *ApaI* mutation in *VDR* (rs7975232) also associates with risk of asthma (196), and rs17470271 in *CYP27A1* has been found to associate with another spirometric lung function index of asthma severity - FEV_1 (297). Several reports have also been made of vitamin D pathway SNP modifying the effects of vitamin D supplementation on risk of disease outcomes: *TaqI* mutation (rs731236) in *VDR* has been shown to modify the effects of vitamin D supplementation on time to sputum culture conversion in patients receiving treatment for tuberculosis, in the UK (205); risk of advanced colorectal adenoma, in another UK clinical trial (341); and *FokI* mutation has been reported to modify the effect of vitamin D supplementation on treatment of Parkinson's disease, in a clinical trial conducted in Japan (342).

To my knowledge, an investigation of the effects of genetic variation on response to vitamin D supplementation for the prevention of URI or asthma exacerbation in adult patients has not been

conducted. I therefore conducted main effect and effect-modification analyses on my panel of 35 SNP in 11 vitamin D pathway genes (described in Methods, section 2.3.2) and the previously mentioned respiratory outcomes.

7.2. Results.

7.2.1. Main effects: does vitamin D pathway genotype influence risk of URI or asthma exacerbation, independent of vitamin D supplementation?

Results of the main effects analyses are presented in Table 7.1 and Table 7.2. Multivariable Cox regression analysis (described in Methods, section 2.4.2) identified the following SNP genotypes to associate with risk of URI: referent to GG genotype for rs2762934 (*CYP24A1*), AG genotype associates with a 21% lower risk, and AA genotype associates with a 65% lower risk (P value for trend = 0.022); referent to CC genotype for rs7041 (*DBP*), AC genotype associates with a 28% lower risk, and AA genotype associates with a 42% lower risk (P for trend = 0.012); referent to GG genotype for rs4588 (*DBP*), GT genotype associates with a 22% lower risk, and TT genotype associates with a 57% lower risk (P for trend = 0.015); referent to TT genotype for rs16846876 (*DBP*), AT genotype associates with a 18% lower risk, and TT genotype associates with a 55% lower risk (P value for trend = 0.033). After correction for multiple comparison testing (Bejamini & Hochberg method with a false discovery rate of 5%) these associations did not remain significant. Multivariable Cox regression analysis did not identify any SNP that associate with risk of asthma exacerbation (Table 7.2).

Table 7.1. Main effects: Time to first URI.

Gene	SNP	Genotype	N	Days at risk (IQR)	Univariable Hazard Ratio (95% CI)	P value	Multivariable Hazard Ratio (95% CI) [1]	P value
CUBN	rs3740165	TT	221	121 (39 to UD)	referent	0.82	referent	0.70
		TC	22	156 (97 to 229)	1.06 (0.65 to 1.73)		1.10 (0.67 to 1.81	
		CC	0*	-	-		-	
CYP24A1	rs2762939	GG	135	110 (40 to 304)	referent	0.28	referent	0.12
		CG	91	128 (36 to UD)	0.86 (0.62 to 1.18)		0.81 (0.58 to 1.12)	
		CC	17	178 (46 to UD)	0.79 (0.42 to 1.47)		0.69 (0.37 to 1.31)	
	rs2248137	CC	84	106 (41 to 344)	referent	0.50	referent	0.51
		CG	110	144 (34 to 315)	1.00 (0.72 to 1.41)		0.95 (0.68 to 1.33)	
		GG	49	138 (60 to UD)	0.85 (0.55 to 1.30)		0.83 (0.53 to 1.28)	
	rs2762934	GG	175	112 (36 to 304)	referent	0.042	referent	0.022
		AG	65	158 (53 to UD)	0.81 (0.57 to 1.14)		0.79 (0.55 to 1.11)	
		AA	6	UD (178 to UD)	0.28 (0.07 to 1.15)		0.25 (0.06 to 1.01)	
	rs6013897	TT	148	128 (38 to UD)	referent	0.16	referent	0.12
		AT	82	138 (46 to 315)	1.10 (0.79 to 1.51)		1.11 (0.80 to 1.53)	
		AA	13	90 (35 to 156)	1.70 (0.93 to 3.09)		1.87 (0.99 to 3.53)	
CYP27A1	rs17470271	AA	97	106 (36 to 339)	referent	0.35	referent	0.54
		AT	113	128 (40 to UD)	0.88 (0.63 to 1.21)		0.89 (0.64 to 1.23)	
		TT	37	165 (82 to UD)	0.83 (0.53 to 1.29)		0.90 (0.57 to 1.42)	
CYP27B1	rs4646537	TT	223	121 (41 to 339)	referent	0.31	referent	0.35
		GT	22	180 (35 to UD)	0.75 (0.42 to 1.31		0.75 (0.41 to 1.36)	
		GG	0*	-	-		-	
	rs4646536	AA	114	130 (42 to UD)	referent	0.23	referent	0.11
		AG	100	128 (38 to 328)	1.18 (0.85 to 1.62)		1.27 (0.91 to 1.78)	
		GG	26	121 (41 to 250)	1.28 (0.77 to 2.12)		1.41 (0.83 to 2.39)	
CYP2R1	rs10500804	TT	84	121 (46 to UD)	referent	1.00	referent	0.95
		GT	126	138 (40 to 346)	0.98 (0.71 to 1.36)		1.00 (0.71 to 1.40)	
		GG	36	94 (33 to UD)	1.01 (0.64 to 1.60)		1.02 (0.63 to 1.64)	
	rs2060793	GG	92	108 (41 to 344)	referent	0.73	referent	0.83
		AG	112	138 (39 to UD)	0.95 (0.69 to 1.31)		0.97 (0.69 to 1.35)	
		AA	33	130 (67 to UD)	0.93 (0.58 to 1.50)		0.96 (0.59 to 1.56)	
	rs10766197	GG	72	128 (46 to UD)	referent	0.40	referent	0.62
		AG	126	120 (38 to 344)	1.08 (0.77 to 1.53)		1.13 (0.80 to 1.61)	
		AA	35	90 (40 to UD)	1.08 (0.67 to 1.75)		1.09 (0.66 to 1.81)	
CYP3A4	rs2740574	AA	213	134 (42 to UD)	referent	0.010	referent	0.14
		AG	25	53 (32 to 180)	1.69 (1.05 to 2.69)		1.65 (1.02 to 2.69)	
		GG	9	71 (19 to 138)	1.85 (0.91 to 3.78)		1.24 (0.55 to 2.80)	
DBP	rs7041	CC	75	90 (36 to 287)	referent	0.099	referent	0.012
		AC	113	165 (42 to UD)	0.79 (0.56 to 1.11)		0.72 (0.51 to 1.02)	
		AA	54	166 (50 to UD)	0.71 (0.47 to 1.08)		0.58 (0.37 to 0.90)	
	rs4588	GG	133	106 (39 to 285)	referent	0.012	referent	0.015
		GT	97	180 (41 to UD)	0.77 (0.57 to 1.06)		0.78 (0.57 to 1.08)	
		TT	15	335 (166 to UD)	0.43 (0.20 to 0.93)		0.43 (0.20 to 0.93)	
	rs12512631	TT	95	158 (42 to UD)	referent	0.14	referent	0.053
		CT	117	128 (53 to 328)	1.14 (0.82 to 1.58)		1.25 (0.89 to 1.74)	
		CC	34	110 (30 to 309)	1.42 (0.90 to 2.23)		1.54 (0.97 to 2.45)	
	rs2070741	TT	204	128 (42 to UD)	referent	0.14	referent	0.13
		TG	39	62 (33 to 229)	1.35 (0.91 to 2.00)		1.36 (0.91 to 2.03)	
		GG	0*	-	-		-	
	rs2298849	AA	154	125 (39 to UD)	referent	0.49	referent	0.92
		AG	83	106 (40 to 229)	1.27 (0.93 to 1.74)		1.25 (0.91 to 1.71)	
		GG	8	204 (21 to UD)	0.74 (0.30 to 1.81)		0.45 (0.17 to 1.15)	
	rs16846876	AA	116	110 (38 to 315)	referent	0.097	referent	0.033
		AT	111	125 (43 to UD)	0.88 (0.65 to 1.20)		0.82 (0.59 to 1.13)	
		TT	14	262 (166 to UD)	0.50 (0.23 to 1.09)		0.45 (0.21 to 0.99)	
DHCR7	rs3829251	GG	186	144 (41 to UD)	referent	0.059	referent	0.079

Table 7.1 continued.

Gene	SNP	Genotype	N	Days at risk (IQR)	Univariable Hazard Ratio (95% CI)	P value	Multivariable Hazard Ratio (95% CI) [1]	P value
		AG	58	78 (36 to 206)	1.42 (1.01 to 2.00)		1.38 (0.97 to 1.97)	
		AA	2*	-	-		-	
	rs12785878	TT	131	144 (40 to UD)	referent	0.35	referent	0.28
		GT	84	124 (43 to 309)	1.10 (0.79 to 1.52)		1.18 (0.84 to 1.65)	
		GG	31	78 (17 to 346)	1.23 (0.78 to 1.94)		1.24 (0.76 to 2.04)	
LRP2	rs3755166	GG	84	144 (54 to UD)	referent	0.57	referent	0.66
		AG	126	121 (36 to 285)	1.21 (0.87 to 1.68)		1.15 (0.82 to 1.61)	
		AA	36	97 (33 to UD)	1.05 (0.65 to 1.70)		1.05 (0.64 to 1.72)	
RXRA	rs7861779	GG	154	110 (35 to 346)	referent	0.90	referent	0.80
		GA	75	178 (62 to UD)	0.84 (0.60 to 1.16)		0.83 (0.59 to 1.17)	
		AA	15	75 (32 to 130)	1.57 (0.86 to 2.85)		1.25 (0.67 to 2.32)	
	rs9409929	GG	106	108 (42 to 315)	referent	0.53	referent	0.88
		AG	111	158 (38 to UD)	0.89 (0.65 to 1.22)		0.97 (0.70 to 1.34)	
		AA	28	120 (36 to UD)	0.91 (0.55 to 1.48)		0.97 (0.59 to 1.61)	
VDR	rs4334089	GG	124	112 (36 to UD)	referent	0.49	referent	0.75
		AG	90	144 (50 to UD)	1.00 (0.72 to 1.38)		0.85 (0.61 to 1.19)	
		AA	31	110 (39 to 269)	1.22 (0.78 to 1.92)		1.25 (0.79 to 1.98)	
	rs10783219	AA	113	125 (41 to 285)	referent	0.85	referent	0.92
		AT	100	134 (59 to UD)	0.77 (0.55 to 1.06)		0.75 (0.54 to 1.05)	
		TT	31	84 (33 to 335)	1.18 (0.75 to 1.86)		1.22 (0.77 to 1.94)	
	rs4516035	TT	99	86 (34 to 328)	referent	0.28	referent	0.44
		CT	101	177 (63 to UD)	0.73 (0.52 to 1.02)		0.84 (0.59 to 1.19)	
		CC	43	127 (41 to UD)	0.87 (0.57 to 1.33)		0.88 (0.56 to 1.36)	
	rs11568820	CC	136	128 (41 to UD)	referent	0.043	referent	0.11
		CT	71	166 (40 to UD)	1.00 (0.70 to 1.41)		0.96 (0.67 to 1.36)	
		TT	32	71 (33 to 170)	1.76 (1.15 to 2.70)		1.68 (1.06 to 2.67)	
	rs7976091	CC	138	128 (42 to UD)	referent	0.081	referent	0.28
		CT	72	158 (40 to UD)	1.03 (0.73 to 1.45)		0.98 (0.69 to 1.39)	
		TT	31	75 (34 to 170)	1.62 (1.04 to 2.50)		1.42 (0.89 to 2.26)	
	rs2238136	CC	142	110 (40 to 309)	referent	0.64	referent	0.83
		CT	85	165 (54 to UD)	0.79 (0.57 to 1.10)		0.81 (0.58 to 1.13)	
		TT	19	67 (31 to 262)	1.17 (0.68 to 2.01)		1.27 (0.73 to 2.20)	
	rs1544410	CC	85	170 (47 to UD)	referent	0.17	referent	0.38
		CT	116	110 (34 to 315)	1.31 (0.93 to 1.84)		1.22 (0.86 to 1.75)	
		TT	42	121 (42 to 299)	1.29 (0.84 to 2.00)		1.18 (0.75 to 1.86)	
	rs2228570	GG	102	144 (47 to 346)	referent	0.70	referent	0.57
		AG	111	106 (39 to UD)	1.07 (0.78 to 1.48)		1.04 (0.75 to 1.44)	
		AA	34	128 (30 to 335)	1.07 (0.67 to 1.69)		1.15 (0.72 to 1.84)	
	rs2853559	GG	103	130 (42 to UD)	referent	0.77	referent	0.48
		AG	108	124 (39 to 328)	1.15 (0.83 to 1.58)		1.23 (0.89 to 1.71)	
		AA	35	125 (40 to UD)	0.99 (0.62 to 1.59)		1.07 (0.66 to 1.75)	
	rs7975232	AA	75	138 (53 to 346)	referent	0.60	referent	0.81
		AC	111	106 (36 to UD)	1.06 (0.75 to 1.50)		0.98 (0.68 to 1.39)	
		CC	51	128 (47 to UD)	0.87 (0.56 to 1.34)		0.95 (0.60 to 1.48)	
	rs7970314	AA	138	127 (41 to UD)	referent	0.18	referent	0.39
		AG	74	128 (39 to 344)	1.03 (0.74 to 1.44)		0.98 (0.70 to 1.38)	
		GG	34	82 (34 to 210)	1.41 (0.92 to 2.17)		1.31 (0.83 to 2.08)	
	rs731236	AA	95	120 (42 to UD)	referent	0.63	referent	0.84
		AG	114	129 (34 to 309)	1.13 (0.81 to 1.56)		1.07 (0.76 to 1.50)	
		GG	34	112 (53 to 346)	1.07 (0.67 to 1.70)		1.02 (0.63 to 1.67)	

[1] *Adjusted for stratification factors i.e. British Thoracic Society treatment step (2/3 vs. 4/5) and inclusion in vs. exclusion from sputum induction sub-study; significant predictors of upper respiratory infection i.e. Body mass index (<30 kg/m2 vs. ≥30 kg/m2), exhaled nitric oxide level (<26 parts per billion vs. ≥26 parts per billion), and sex; and allocation to study intervention or placebo arm. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for interaction remain significant.*

******* *Genotype could not be analysed due to <5 participants and/or too few events within the sub category.*

Abbreviations: SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, UD: Undefined, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor.

Table 7.2. Main effects: Time to first asthma exacerbation.

Gene	SNP	Genotype	N	Days at risk (IQR)	Univariable Hazard Ratio (95% CI)	P value	Multivariable Hazard Ratio (95% CI) [1]	P value
CUBN	rs3740165	TT	221	UD (151 to UD)	referent	0.50	referent	0.76
		TC	22	UD (98 to UD)	1.25 (0.65 to 2.41)		1.11 (0.57 to 2.14)	
		CC	0*	-	-		-	
CYP24A1	rs2762939	GG	135	UD (213 to UD)	referent	0.96	referent	0.50
		CG	91	UD (144 to UD)	1.04 (0.68 to 1.60)		0.89 (0.58 to 1.37)	
		CC	17	UD (63 to UD)	0.95 (0.41 to 2.21)		0.79 (0.33 to 1.89)	
	rs2248137	CC	84	UD (144 to UD)	referent	0.74	referent	0.50
		CG	110	UD (158 to UD)	1.07 (0.69 to 1.68)		1.01 (0.64 to 1.58)	
		GG	49	UD (192 to UD)	0.87 (0.49 to 1.57)		0.79 (0.44 to 1.43)	
	rs2762934	GG	175	UD (141 to UD)	referent	0.52	referent	0.68
		AG	65	UD (251 to UD)	0.78 (0.48 to 1.25)		0.82 (0.51 to 1.33)	
		AA	6	300 (43 to UD)	1.27 (0.40 to 4.02)		1.33 (0.41 to 4.35)	
	rs6013897	TT	148	UD (202 to UD)	referent	0.12	referent	0.17
		AT	82	UD (141 to UD)	1.09 (0.71 to 1.69)		1.09 (0.71 to 1.69)	
		AA	13	218 (37 to UD)	2.26 (1.07 to 4.75)		1.98 (0.94 to 4.21)	
CYP27A1	rs17470271	AA	97	UD (136 to UD)	referent	0.42	referent	0.84
		AT	113	UD (202 to UD)	0.86 (0.56 to 1.33)		0.91 (0.59 to 1.40)	
		TT	37	UD (155 to UD)	0.80 (0.43 to 1.50)		0.99 (0.53 to 1.88)	
CYP27B1	rs4646537	TT	223	UD (186 to UD)	referent	0.26	referent	0.26
		GT	22	301 (58 to UD)	1.44 (0.77 to 2.70)		1.46 (0.75 to 2.82)	
		GG	0*	-	-		-	
	rs4646536	AA	114	UD (216 to UD)	referent	0.71	referent	0.81
		AG	100	UD (114 to UD)	1.37 (0.90 to 2.09)		1.15 (0.75 to 1.77)	
		GG	26	UD (274 to UD)	0.80 (0.36 to 1.77)		0.92 (0.41 to 2.07)	
CYP2R1	rs10500804	TT	84	UD (117 to UD)	referent	0.077	referent	0.058
		GT	126	UD (155 to UD)	0.85 (0.56 to 1.30)		0.78 (0.50 to 1.19)	
		GG	36	UD (270 to UD)	0.52 (0.26 to 1.05)		0.52 (0.25 to 1.06)	
	rs2060793	GG	92	UD (231 to UD)	referent	0.068	referent	0.057
		AG	112	UD (105 to UD)	1.74 (1.10 to 2.74)		1.73 (1.09 to 2.74)	
		AA	33	UD (151 to UD)	1.50 (0.79 to 2.85)		1.58 (0.82 to 3.02)	
	rs10766197	GG	72	UD (117 to UD)	referent	0.13	referent	0.11
		AG	126	UD (155 to UD)	0.76 (0.49 to 1.19)		0.72 (0.46 to 1.12)	
		AA	35	UD (233 to UD)	0.63 (0.33 to 1.23)		0.63 (0.32 to 1.23)	
CYP3A4	rs2740574	AA	213	UD (155 to UD)	referent	0.64	referent	0.26
		AG	25	UD (63 to UD)	1.29 (0.69 to 2.42)		1.43 (0.75 to 2.74)	
		GG	9*	-	-		-	
DBP	rs7041	CC	75	364 (119 to UD)	referent	0.30	referent	0.13
		AC	113	UD (221 to UD)	0.61 (0.38 to 0.97)		0.58 (0.36 to 0.92)	
		AA	54	UD (213 to UD)	0.80 (0.47 to 1.37)		0.70 (0.41 to 1.21)	
	rs4588	GG	133	UD (136 to UD)	referent	0.66	referent	0.61
		GT	97	UD (158 to UD)	0.96 (0.63 to 1.46)		0.87 (0.56 to 1.35)	
		TT	15	UD (274 to UD)	0.79 (0.32 to 1.98)		0.93 (0.37 to 2.36)	
	rs12512631	TT	95	UD (218 to UD)	referent	0.34	referent	0.16
		CT	117	UD (126 to UD)	1.34 (0.86 to 2.07)		1.43 (0.92 to 2.22)	
		CC	34	UD (151 to UD)	1.22 (0.65 to 2.27)		1.39 (0.74 to 2.61)	
	rs2070741	TT	204	UD (151 to UD)	referent	0.85	referent	0.78
		TG	39	UD (103 to UD)	1.05 (0.62 to 1.80)		1.08 (0.63 to 1.85)	
		GG	0*	-	-		-	
	rs2298849	AA	154	UD (158 to UD)	referent	0.93	referent	0.89
		AG	83	UD (133 to UD)	1.19 (0.78 to 1.81)		1.21 (0.79 to 1.87)	
		GG	8	UD (241 to UD)	0.53 (0.13 to 2.18)		0.53 (0.13 to 2.19)	
	rs16846876	AA	116	UD (135 to UD)	referent	0.41	referent	0.38
		AT	111	UD (155 to UD)	0.92 (0.61 to 1.39)		0.91 (0.60 to 1.37)	
		TT	14	UD (274 to UD)	0.63 (0.23 to 1.74)		0.62 (0.22 to 1.73)	
DHCR7	rs3829251	GG	186	UD (186 to UD)	referent	0.064	referent	0.18

Table 7.2 continued.

Gene	SNP	Genotype	N	Days at risk (IQR)	Univariable Hazard Ratio (95% CI)	P value	Multivariable Hazard Ratio (95% CI) [1]	P value
		AG	58	UD (155 to UD)	1.32 (0.84 to 2.07)		1.16 (0.72 to 1.85)	
		AA	2*	-	-		-	
	rs12785878	TT	131	UD (216 to UD)	referent	0.54	referent	0.95
		GT	84	UD (114 to UD)	1.28 (0.83 to 1.98)		1.25 (0.81 to 1.94)	
		GG	31	UD (203 to UD)	1.05 (0.54 to 2.02)		0.86 (0.43 to 1.69)	
LRP2	rs3755166	GG	84	UD (244 to UD)	referent	0.20	referent	0.41
		AG	126	353 (119 to UD)	1.90 (1.18 to 3.05)		1.64 (1.01 to 2.66)	
		AA	36	UD (218 to UD)	1.18 (0.58 to 2.41)		1.09 (0.53 to 2.25)	
RXRA	rs7861779	GG	154	UD (151 to UD)	referent	0.78	referent	0.90
		GA	75	UD (118 to UD)	1.16 (0.75 to 1.78)		1.14 (0.73 to 1.77)	
		AA	15	UD (218 to UD)	0.90 (0.36 to 2.24)		0.72 (0.28 to 1.83)	
	rs9409929	GG	106	UD (136 to UD)	referent	0.96	referent	0.90
		AG	111	UD (202 to UD)	0.86 (0.56 to 1.32)		0.93 (0.60 to 1.43)	
		AA	28	UD (133 to UD)	1.16 (0.63 to 2.16)		1.12 (0.60 to 2.09)	
VDR	rs4334089	GG	124	UD (106 to UD)	referent	0.16	referent	0.11
		AG	90	UD (202 to UD)	0.85 (0.55 to 1.30)		0.80 (0.52 to 1.24)	
		AA	31	UD (224 to UD)	0.63 (0.32 to 1.23)		0.60 (0.30 to 1.20)	
	rs10783219	AA	113	UD (202 to UD)	referent	0.22	referent	0.19
		AT	100	UD (213 to UD)	0.86 (0.55 to 1.34)		0.79 (0.50 to 1.24)	
		TT	31	270 (37 to UD)	1.77 (1.01 to 3.09)		2.01 (1.13 to 3.55)	
	rs4516035	TT	99	UD (128 to UD)	referent	0.43	referent	0.54
		CT	101	UD (155 to UD)	0.92 (0.59 to 1.43)		0.96 (0.61 to 1.50)	
		CC	43	UD (224 to UD)	0.78 (0.43 to 1.42)		0.82 (0.45 to 1.49)	
	rs11568820	CC	136	UD (144 to UD)	referent	0.72	referent	0.47
		CT	71	UD (128 to UD)	0.99 (0.63 to 1.57)		0.97 (0.61 to 1.55)	
		TT	32	UD (218 to UD)	0.87 (0.47 to 1.63)		0.76 (0.40 to 1.46)	
	rs7976091	CC	138	UD (136 to UD)	referent	0.43	referent	0.26
		CT	72	UD (128 to UD)	0.92 (0.58 to 1.44)		0.91 (0.57 to 1.44)	
		TT	31	UD (221 to UD)	0.77 (0.41 to 1.48)		0.67 (0.34 to 1.30)	
	rs2238136	CC	142	UD (135 to UD)	referent	0.97	referent	0.74
		CT	85	UD (220 to UD)	0.62 (0.39 to 1.00)		0.61 (0.38 to 0.99)	
		TT	19	274 (101 to UD)	1.65 (0.89 to 3.06)		1.43 (0.76 to 2.68)	
	rs1544410	CC	85	UD (155 to UD)	referent	0.31	referent	0.55
		CT	116	UD (192 to UD)	1.07 (0.67 to 1.70)		0.89 (0.56 to 1.44)	
		TT	42	UD (141 to UD)	1.37 (0.78 to 2.42)		1.25 (0.71 to 2.21)	
	rs2228570	GG	102	UD (216 to UD)	referent	0.85	referent	0.28
		AG	111	UD (119 to UD)	1.11 (0.73 to 1.71)		1.30 (0.84 to 2.01)	
		AA	34	UD (269 to UD)	1.00 (0.53 to 1.87)		1.29 (0.68 to 2.44)	
	rs2853559	GG	103	UD (126 to UD)	referent	0.79	referent	0.77
		AG	108	UD (229 to UD)	0.71 (0.46 to 1.10)		0.68 (0.43 to 1.06)	
		AA	35	UD (117 to UD)	1.12 (0.63 to 1.99)		1.16 (0.64 to 2.09)	
	rs7975232	AA	75	UD (151 to UD)	referent	0.98	referent	0.94
		AC	111	UD (216 to UD)	0.73 (0.45 to 1.16)		0.72 (0.44 to 1.17)	
		CC	51	UD (118 to UD)	1.05 (0.62 to 1.79)		1.08 (0.63 to 1.86)	
	rs7970314	AA	138	UD (144 to UD)	referent	0.72	referent	0.55
		AG	74	UD (128 to UD)	0.95 (0.61 to 1.49)		0.96 (0.61 to 1.52)	
		GG	34	UD (218 to UD)	0.90 (0.49 to 1.65)		0.81 (0.43 to 1.52)	
	rs731236	AA	95	UD (203 to UD)	referent	0.47	referent	0.57
		AG	114	UD (213 to UD)	1.00 (0.64 to 1.57)		0.93 (0.59 to 1.46)	
		GG	34	UD (141 to UD)	1.31 (0.72 to 2.36)		1.29 (0.71 to 2.35)	

[1] *Adjusted for stratification factors i.e. British Thoracic Society treatment step (2/3 vs. 4/5) and inclusion in vs. exclusion from sputum induction sub-study; significant predictors of asthma exacerbation i.e. Baseline asthma control test score (<19 vs. ≥19), reversibility assessment (yes vs. no), and sex; and allocation to study intervention or placebo arm. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for interaction remain significant. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category.*

Abbreviations: SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, CI: Confidence interval, UD: Undefined, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor.

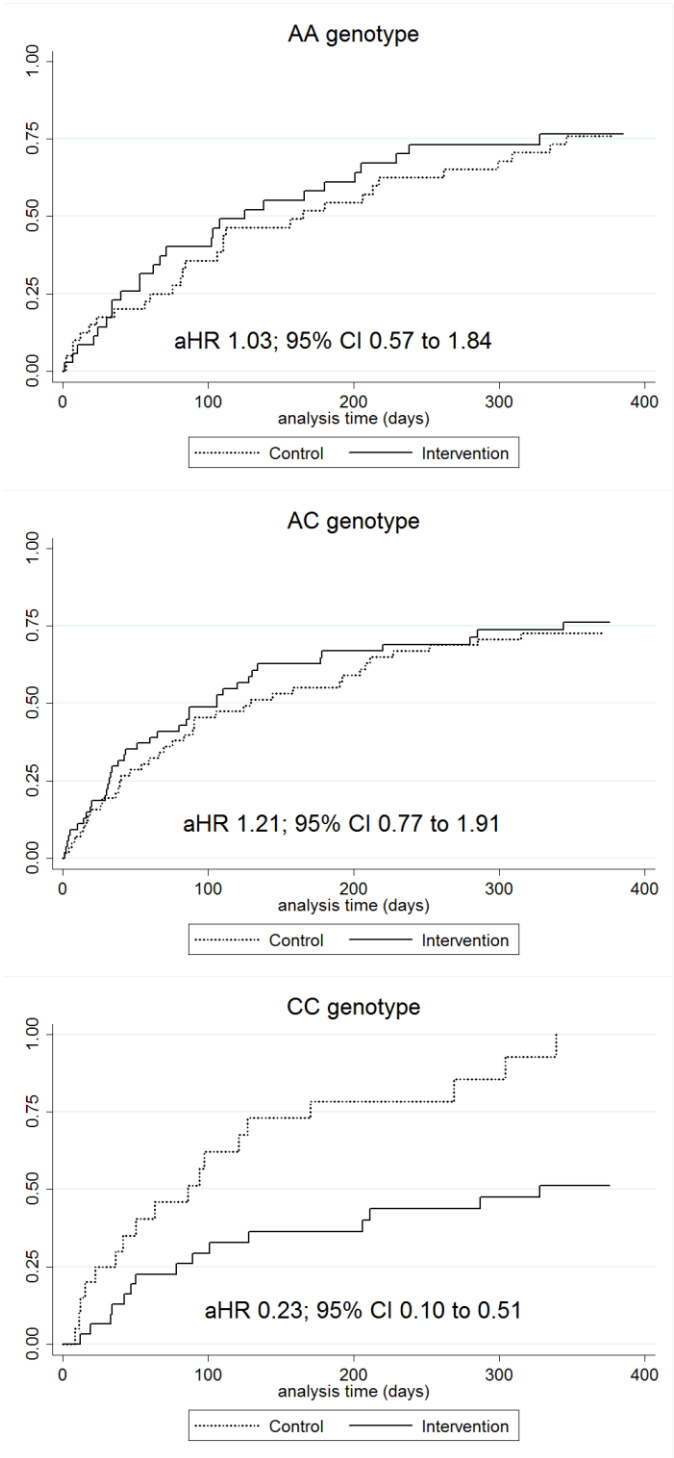
7.2.2. *Interaction analysis: does vitamin D pathway genotype influence effect of vitamin D supplementation in prevention of URI or asthma exacerbation?*

Results of interaction analyses testing for effect modification are presented in Table 7.3 and Table 7.4. Multivariable Cox regression analysis (described in Methods, section 2.4.3) identified 3 SNP in *VDR* which modify the effect of vitamin D supplementation on risk of URI. To illustrate this concept, Figure 7.1 shows Kaplan-Meier failure estimates for time to first URI, stratified by genotype for rs7975232: In participants with CC genotype for rs7975232, vitamin D supplementation offered protection from URI (adjusted hazard ratio [aHR] 0.23; 95% CI 0.10 to 0.51), but had no effect in those with AC genotype (aHR 1.21; 95% CI 0.77 to 1.91), or AA genotype (aHR 1.03; 95% CI 0.57 to 1.84 - adjusted ratio of hazard ratios [aRHR] 0.58; 95% CI 0.37 to 0.91; P value for interaction = 0.017). In participants with CC genotype for rs1544410, vitamin D supplementation offered protection from URI (aHR 0.54; 95% CI 0.31 to 0.94), but had no effect in those with CT genotype (aHR 1.10; 95% CI 0.71 to 1.71), or TT genotype (aHR 1.77; 95% CI 0.78 to 4.06 - aRHR 1.54; 95% CI 1.00 to 2.36; P for interaction = 0.049). Finally, in participants with AA genotype for rs731236, vitamin D supplementation offered protection from URI (aHR 0.51; 95% CI 0.31 to 0.85), but had no effect in those with AG genotype (aHR 1.23; 95% CI 0.79 to 1.92), or GG genotype (aHR 1.60; 95% CI 0.68 to 3.76 - aRHR 1.68; 95% CI 1.08 to 2.61; P value for interaction = 0.022).

Multivariable Cox regression analysis identified 1 SNP in *DBP* which modifies the effect of vitamin D supplementation on risk of asthma exacerbation. In participants with CC genotype for rs12512631, vitamin D supplementation offered protection from asthma exacerbation (aHR 0.47; 95% CI 0.15 to 1.47), but had no effect in those with CT genotype (0.77; 95% CI 0.44 to 1.37), or TT genotype (aHR 2.14; 95% CI 1.03 to 4.45; P value for interaction = 0.005).

After correction for multiple comparisons testing (Bejamini & Hochberg method with a false discovery rate of 5%) none of the observed associations between SNP and the effects of vitamin D supplementation on the risk of URI or asthma exacerbations remained significant.

Figure.7.1. Kaplan-Meier failure estimates from the effect modification analysis, illustrating the effect of allocation on number of days post randomisation to first URI event, stratified by genotype for the VDR SNP, rs7975232. Allocation to the treatment arm did not affect risk of URI for those with AA, or AC genotypes, but for those with the CC genotype, vitamin D offered significant protection.



Interaction term aHR 0.58; 95% CI 0.37 to 0.91; P=0.017

Table.7.3. Interaction analysis: Time to first URI.

Gene	SNP	Geno-type	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within subgroup (95% CI) [1]	Hazard ratio for allocation*geno-type interaction (95% CI) [1]	P value for interaction [1]
CUBN	rs3740165	TT	109	110 (38 to 309)	112	130 (40 to UD)	0.84 (0.61 to 1.16)	0.84 (0.61 to 1.16)	0.29
		TC	11	192 (82 to 315)	11	128 (106 to 229)	0.82 (0.23 to 2.94)		
		CC	0	-	0	-	-		
CYP24A1	rs2762939	GG	68	97 (36 to 252)	67	121 (51 to UD)	0.66 (0.44 to 0.99)	0.73 (0.50 to 1.08)	0.18
		CG	47	124 (50 to UD)	44	128 (31 to UD)	1.39 (0.81 to 2.39)		
		CC	6	156 (46 to 158)	11	287 (34 to UD)	0.48 (0.09 to 2.56)		
	rs2248137	CC	42	106 (41 to 304)	42	108 (47 to UD)	0.86 (0.51 to 1.44)	1.04 (0.68 to 1.59)	0.87
		CG	58	114 (26 to 299)	59	166 (34 to UD)	0.91 (0.57 to 1.44)		
		GG	20	110 (59 to UD)	29	138 (80 to UD)	0.70 (0.31 to 1.55)		
	rs2762934	GG	83	90 (36 to 262)	92	128 (34 to 344)	0.83 (0.58 to 1.17)	1.12 (0.60 to 2.09)	0.72
		AG	34	158 (75 to 309)	31	108 (42 to UD)	0.86 (0.45 to 1.64)		
		AA	4*	-	2*	-	-		
	rs6013897	TT	73	110 (36 to UD)	75	134 (47 to UD)	0.84 (0.56 to 1.25)	0.86 (0.51 to 1.48)	0.60
		AT	37	144 (59 to 315)	45	125 (42 to UD)	0.85 (0.47 to 1.52)		
		AA	11	90 (35 to 156)	2*	-	-		
CYP27A1	rs17470271	AA	48	106 (23 to UD)	49	103 (42 to 287)	1.17 (0.72 to 1.91)	0.79 (0.51 to 1.23)	0.30
		AT	53	105 (41 to 285)	60	138 (34 to UD)	0.75 (0.47 to 1.18)		
		TT	22	165 (82 to 304)	15	125 (43 to UD)	0.91 (0.40 to 2.11)		
CYP27B1	rs4646537	TT	109	110 (40 to 299)	114	128 (42 to UD)	0.83 (0.61 to 1.13)	1.42 (0.43 to 4.73)	0.57
		GT	12	165 (35 to UD)	10	180 (29 to UD)	0.66 (0.10 to 4.13)		
		GG	0	-	0	-	-		
	rs4646536	AA	52	121 (41 to UD)	62	130 (42 to UD)	1.04 (0.64 to 1.67)	0.67 (0.41 to 1.08)	0.099
		AG	50	129 (35 to 335)	50	128 (40 to 328)	0.91 (0.57 to 1.45)		
		GG	20	83 (27 to 211)	6	UD (121 to UD)	0.12 (0.02 to 0.70)		
CYP2R1	rs10500804	TT	36	97 (46 to 217)	48	130 (38 to UD)	0.99 (0.57 to 1.71)	0.79 (0.50 to 1.25)	0.31
		GT	67	144 (38 to 339)	42	128 (42 to UD)	1.03 (0.67 to 1.58)		
		GG	20	75 (26 to 304)	16	110 (33 to UD)	0.53 (0.21 to 1.36)		
	rs2060793	GG	52	97 (40 to 335)	40	110 (42 to UD)	0.92 (0.57 to 1.51)	0.91 (0.58 to 1.43)	0.67
		AG	53	114 (50 to 299)	59	138 (34 to UD)	0.87 (0.55 to 1.37)		
		AA	12	86 (6 to 190)	21	130 (71 to UD)	0.60 (0.23 to 1.57)		
	rs10766197	GG	32	97 (46 to UD)	40	130 (38 to UD)	0.96 (0.53 to 1.75)	0.76 (0.47 to 1.23)	0.27
		AG	65	129 (38 to 309)	61	106 (40 to UD)	0.96 (0.63 to 1.47)		
		AA	21	82 (40 to 335)	14	108 (42 to UD)	0.52 (0.19 to 1.42)		
CYP3A4	rs2740574	AA	106	129 (56 to 346)	107	166 (42 to UD)	0.93 (0.67 to 1.29)	0.94 (0.52 to 1.69)	0.84
		AG	12	35 (11 to 50)	13	128 (53 to UD)	0.35 (0.10 to 1.15)		
		GG	5	66 (12 to 204)	4*	-	-		
DBP	rs7041	CC	37	86 (40 to 206)	38	106 (34 to 344)	0.76 (0.42 to 1.35)	1.24 (0.80 to 1.93)	0.34
		AC	57	180 (38 to 346)	56	128 (47 to UD)	0.89 (0.56 to 1.42)		
		AA	28	105 (46 to UD)	26	166 (53 to UD)	0.88 (0.43 to 1.79)		
	rs4588	GG	62	90 (39 to 269)	71	108 (40 to 287)	0.87 (0.58 to 1.31)	0.98 (0.58 to 1.65)	0.95
		GT	54	170 (38 to 339)	43	201 (43 to UD)	0.68 (0.41 to 1.13)		
		TT	6	335 (262 to UD)	9	UD (166 to UD)	0.53 (0.04 to 6.72)		
	rs12512631	TT	49	165 (50 to UD)	46	108 (40 to UD)	1.08 (0.65 to 1.80)	0.68 (0.44 to 1.07)	0.099
		CT	57	112 (41 to 285)	60	130 (53 to UD)	0.83 (0.54 to 1.29)		
		CC	17	59 (26 to 213)	17	205 (34 to 328)	0.56 (0.21 to 1.52)		
	rs2070741	TT	105	112 (41 to 304)	99	138 (50 to UD)	0.78 (0.56 to 1.09)	0.71 (0.31 to 1.65)	0.43
		TG	15	50 (11 to UD)	24	62 (34 to 211)	0.99 (0.44 to 2.24)		
		GG	0	-	0	-	-		
	rs2298849	AA	72	121 (38 to 335)	82	128 (42 to UD)	0.81 (0.56 to 1.20)	1.49 (0.88 to 2.52)	0.14
		AG	44	105 (36 to 208)	39	108 (40 to 285)	0.70 (0.42 to 1.18)		
		GG	6	269 (204 to UD)	2*	-	-		
	rs16846876	AA	54	110 (36 to 315)	62	120 (40 to 328)	0.79 (0.50 to 1.25)	0.94 (0.55 to 1.60)	0.82
		AT	60	129 (46 to 335)	51	108 (34 to UD)	0.77 (0.49 to 1.23)		
		TT	8	250 (90 to UD)	6	UD (166 to UD)	1.66 (0.15 to 18.91)		

Table 7.3 continued.

Gene	SNP	Geno-type	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within subgroup (95% CI) [1]	Hazard ratio for allocation*geno-type interaction (95% CI) [1]	P value for interaction [1]
DHCR7	rs3829251	GG	92	144 (40 to 339)	94	166 (43 to UD)	0.85 (0.60 to 1.21)	1.31 (0.67 to 2.56)	0.44
		AG	28	75 (19 to 192)	30	78 (42 to 328)	1.11 (0.58 to 2.11)		
		AA	1*	-	1*	-	-		
	rs12785878	TT	72	112 (40 to 335)	59	178 (38 to UD)	0.74 (0.48 to 1.15)	1.06 (0.68 to 1.65)	0.81
		GT	37	158 (69 to UD)	47	89 (42 to 287)	1.40 (0.80 to 2.44)		
		GG	13	46 (12 to 204)	18	121 (33 to UD)	0.50 (0.17 to 1.46)		
LRP2	rs3755166	GG	45	156 (60 to 315)	39	138 (42 to UD)	0.74 (0.43 to 1.28)	0.97 (0.62 to 1.52)	0.89
		AG	60	110 (36 to 285)	66	130 (34 to 287)	0.92 (0.60 to 1.40)		
		AA	17	97 (18 to UD)	19	125 (53 to UD)	0.36 (0.13 to 1.01)		
RXRA	rs7861779	GG	74	105 (35 to 269)	80	120 (34 to UD)	0.78 (0.53 to 1.14)	1.12 (0.67 to 1.87)	0.66
		GA	37	180 (66 to UD)	38	177 (62 to UD)	1.03 (0.58 to 1.83)		
		AA	9	75 (36 to 110)	6	71 (32 to 138)	1.08 (0.27 to 4.36)		
	rs9409929	GG	53	110 (40 to 315)	53	106 (51 to UD)	0.97 (0.61 to 1.54)	0.87 (0.55 to 1.37)	0.54
		AG	55	129 (41 to 285)	56	166 (34 to UD)	0.78 (0.49 to 1.24)		
		AA	14	89 (36 to UD)	14	130 (50 to UD)	0.44 (0.15 to 1.29)		
VDR	rs4334089	GG	62	105 (35 to 315)	62	205 (42 to UD)	0.61 (0.38 to 0.98)	1.25 (0.79 to 1.96)	0.34
		AG	42	190 (60 to UD)	48	120 (38 to 328)	1.28 (0.76 to 2.18)		
		AA	18	82 (41 to 269)	13	166 (33 to 229)	0.79 (0.32 to 1.92)		
	rs10783219	AA	54	94 (39 to 208)	59	166 (50 to UD)	0.67 (0.43 to 1.05)	1.11 (0.69 to 1.76)	0.67
		AT	52	211 (63 to UD)	48	128 (34 to UD)	1.15 (0.69 to 1.91)		
		TT	16	84 (26 to 262)	15	67 (33 to UD)	0.58 (0.23 to 1.45)		
	rs4516035	TT	51	90 (36 to 335)	48	71 (32 to 328)	1.18 (0.74 to 1.88)	0.70 (0.46 to 1.07)	0.10
		CT	47	165 (63 to UD)	54	177 (65 to UD)	0.88 (0.54 to 1.43)		
		CC	21	90 (18 to 190)	22	180 (53 to UD)	0.40 (0.17 to 0.95)		
	rs11568820	CC	69	110 (38 to 315)	67	178 (50 to UD)	0.71 (0.46 to 1.09)	1.22 (0.79 to 1.88)	0.37
		CT	35	206 (40 to UD)	36	120 (40 to UD)	1.10 (0.62 to 1.95)		
		TT	15	75 (39 to 156)	17	71 (33 to 210)	0.94 (0.40 to 2.18)		
	rs7976091	CC	69	110 (38 to 315)	69	178 (53 to UD)	0.69 (0.45 to 1.06)	1.41 (0.92 to 2.17)	0.12
		CT	38	206 (40 to UD)	34	110 (40 to 344)	1.17 (0.66 to 2.11)		
		TT	15	82 (46 to 170)	16	60 (32 to 138)	1.82 (0.73 to 4.53)		
	rs2238136	CC	70	127 (50 to 315)	72	101 (33 to 287)	1.03 (0.70 to 1.52)	0.67 (0.41 to 1.10)	0.11
		CT	45	124 (36 to 339)	40	180 (87 to UD)	0.78 (0.45 to 1.37)		
		TT	7	35 (16 to 105)	12	106 (34 to UD)	0.36 (0.10 to 1.33)		
	rs1544410	CC	40	89 (40 to 269)	45	285 (89 to UD)	0.54 (0.31 to 0.94)	1.54 (1.00 to 2.36)	0.049
		CT	59	124 (35 to 315)	57	106 (34 to 328)	1.10 (0.71 to 1.71)		
		TT	22	156 (75 to 335)	20	67 (34 to 238)	1.77 (0.78 to 4.06)		
	rs2228570	GG	51	144 (41 to 309)	51	166 (50 to UD)	0.80 (0.50 to 1.26)	0.99 (0.64 to 1.53)	0.95
		AG	58	110 (41 to UD)	53	89 (34 to UD)	1.16 (0.73 to 1.83)		
		AA	14	81 (18 to 262)	20	128 (30 to UD)	0.80 (0.29 to 2.20)		
	rs2853559	GG	54	156 (46 to UD)	49	130 (42 to UD)	1.05 (0.65 to 1.70)	0.67 (0.43 to 1.03)	0.066
		AG	50	121 (39 to 285)	58	128 (42 to UD)	0.82 (0.53 to 1.29)		
		AA	19	94 (27 to 208)	16	280 (40 to UD)	0.50 (0.19 to 1.28)		
	rs7975232	AA	40	165 (60 to 346)	35	125 (40 to 328)	1.03 (0.57 to 1.84)	0.58 (0.37 to 0.91)	0.017
		AC	57	129 (40 to UD)	54	106 (32 to 344)	1.21 (0.77 to 1.91)		
		CC	20	86 (22 to 170)	31	328 (78 to UD)	0.23 (0.10 to 0.51)		
	rs7970314	AA	68	105 (35 to 309)	70	177 (53 to UD)	0.70 (0.46 to 1.06)	1.41 (0.93 to 2.15)	0.11
		AG	38	192 (40 to UD)	36	108 (34 to 344)	1.13 (0.64 to 1.98)		
		GG	16	82 (46 to 204)	33	71 (33 to 210)	1.51 (0.65 to 3.51)		
	rs731236	AA	45	83 (36 to 250)	50	211 (78 to UD)	0.51 (0.31 to 0.85)	1.68 (1.08 to 2.61)	0.022
		AG	61	158 (39 to 335)	53	106 (32 to 280)	1.23 (0.79 to 1.92)		
		GG	15	112 (75 to UD)	19	121 (34 to 238)	1.60 (0.68 to 3.76)		

[1] *Adjusted for stratification factors i.e. British Thoracic Society treatment step (2/3 vs. 4/5) and inclusion in vs. exclusion from sputum induction sub-study; significant predictors of upper respiratory infection i.e. Body mass index (<30 kg/m2 vs. ≥30 kg/m2), exhaled nitric oxide level (<26 parts per billion vs. ≥26 parts per billion), and sex; and allocation to study intervention or placebo arm. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for interaction remain significant. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category.*

Abbreviations: SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, CI: Confidence interval, UD: Undefined, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor, CUBN: Cubilin.

Table.7.4. Interaction analysis: Time to first asthma exacerbation.

Gene	SNP	Geno-type	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within subgroup (95% CI) [1]	Hazard ratio for allocation*geno-type interaction (95% CI) [1]	P value for interaction
CUBN	rs3740165	TT	109	UD (136 to UD)	112	UD (186 to UD)	1.14 (0.74 to 1.75)	0.49 (0.13 to 1.91)	0.31
		TC	11	218 (76 to UD)	11	UD (216 to UD)	0.76 (0.15 to 3.81)		
		CC	0	-	0	-	-		
CYP24A1	rs2762939	GG	68	UD (216 to UD)	67	UD (155 to UD)	1.19 (0.68 to 2.10)	0.92 (0.46 to 1.82)	0.81
		CG	47	UD (111 to UD)	44	UD (158 to UD)	1.10 (0.55 to 2.18)		
		CC	6	UD (58 to UD)	11	UD (202 to UD)	0.31 (0.01 to 9.64)		
	rs2248137	CC	42	UD (117 to UD)	42	UD (186 to UD)	0.85 (0.41 to 1.76)	0.99 (0.56 to 1.75)	0.97
		CG	58	UD (241 to UD)	59	353 (151 to UD)	1.80 (0.99 to 3.28)		
		GG	20	UD (117 to UD)	29	UD (203 to UD)	0.67 (0.24 to 1.88)		
	rs2762934	GG	83	UD (141 to UD)	92	UD (155 to UD)	1.23 (0.77 to 1.99)	0.42 (0.18 to 0.98)	0.046
		AG	34	UD (119 to UD)	31	UD (328 to UD)	0.71 (0.29 to 1.77)		
		AA	4*	-	2*	-	-		
	rs6013897	TT	73	UD (216 to UD)	75	UD (202 to UD)	1.23 (0.72 to 2.12)	0.63 (0.32 to 1.25)	0.19
		AT	37	UD (126 to UD)	45	UD (151 to UD)	1.00 (0.48 to 2.08)		
		AA	11	136 (35 to 233)	2*	-	-		
CYP27A1	rs17470271	AA	48	UD (136 to UD)	49	UD (133 to UD)	1.25 (0.66 to 2.35)	0.76 (0.42 to 1.38)	0.36
		AT	53	UD (144 to UD)	60	UD (202 to UD)	0.86 (0.46 to 1.60)		
		TT	22	UD (77 to UD)	15	UD (231 to UD)	1.00 (0.30 to 3.34)		
CYP27B1	rs4646537	TT	109	UD (136 to UD)	114	244 (38 to UD)	1.02 (0.66 to 1.57)	1.30 (0.36 to 4.70)	0.69
		GT	12	UD (58 to UD)	10	UD (202 to UD)	1.80 (0.32 to 10.26)		
		GG	0	-	0	-	-		
	rs4646536	AA	52	UD (241 to UD)	62	UD (202 to UD)	1.38 (0.69 to 2.75)	0.83 (0.42 to 1.64)	0.59
		AG	50	300 (111 to UD)	50	UD (128 to UD)	0.79 (0.43 to 1.45)		
		GG	20	UD (29 to UD)	6	UD (274 to UD)	2.05 (0.15 to 28.22)		
CYP2R1	rs10500804	TT	36	UD (117 to UD)	48	UD (101 to UD)	1.06 (0.54 to 2.05)	1.17 (0.63 to 2.17)	0.61
		GT	67	UD (111 to UD)	42	UD (203 to UD)	0.97 (0.55 to 1.70)		
		GG	20	UD (UD to UD)	16	UD (244 to UD)	2.89 (0.63 to 13.29)		
	rs2060793	GG	52	UD (229 to UD)	40	UD (231 to UD)	1.17 (0.53 to 2.59)	1.00 (0.56 to 1.80)	1.00
		AG	53	342 (43 to UD)	59	UD (135 to UD)	0.77 (0.44 to 1.35)		
		AA	12	UD (269 to UD)	21	274 (133 to UD)	2.60 (0.71 to 9.58)		
	rs10766197	GG	32	UD (117 to UD)	40	UD (61 to UD)	1.18 (0.59 to 2.36)	1.15 (0.61 to 2.18)	0.66
		AG	65	UD (111 to UD)	61	UD (216 to UD)	0.91 (0.51 to 1.63)		
		AA	21	UD (233 to UD)	14	328 (158 to UD)	2.37 (0.62 to 9.02)		
CYP3A4	rs2740574	AA	106	UD (141 to UD)	107	UD (186 to UD)	1.21 (0.78 to 1.86)	0.62 (0.26 to 1.50)	0.29
		AG	12	105 (38 to UD)	13	UD (270 to UD)	0.50 (0.11 to 2.17)		
		GG	5	UD (UD to UD)	4*	-	-		
DBP	rs7041	CC	37	288 (117 to UD)	38	UD (151 to UD)	0.67 (0.32 to 1.40)	1.48 (0.81 to 2.68)	0.20
		AC	57	UD (269 to UD)	56	UD (220 to UD)	1.13 (0.57 to 2.26)		
		AA	28	UD (218 to UD)	26	UD (202 to UD)	1.63 (0.64 to 4.18)		
	rs4588	GG	62	UD (119 to UD)	71	UD (192 to UD)	0.81 (0.47 to 1.42)	1.59 (0.79 to 3.20)	0.19
		GT	54	UD (218 to UD)	43	UD (155 to UD)	1.91 (0.95 to 3.83)		
		TT	6	UD (280 to UD)	9	UD (274 to UD)	1.78 (0.14 to 22.54)		
	rs12512631	TT	49	UD (280 to UD)	46	UD (213 to UD)	2.14 (1.03 to 4.45)	0.44 (0.24 to 0.78)	0.005
		CT	57	UD (117 to UD)	60	UD (128 to UD)	0.77 (0.44 to 1.37)		
		CC	17	UD (36 to UD)	17	UD (317 to UD)	0.47 (0.15 to 1.47)		
	rs2070741	TT	105	UD (119 to UD)	99	UD (192 to UD)	0.87 (0.56 to 1.36)	0.31 (0.08 to 1.19)	0.088
		TG	15	UD (UD to UD)	24	301 (98 to UD)	3.45 (0.90 to 13.29)		
		GG	0	-	0	-	-		
	rs2298849	AA	72	UD (117 to UD)	82	UD (216 to UD)	0.92 (0.54 to 1.55)	1.50 (0.74 to 3.07)	0.26
		AG	44	UD (119 to UD)	39	UD (135 to UD)	1.03 (0.51 to 2.05)		
		GG	6	UD (UD to UD)	2*	-	-		
	rs16846876	AA	54	UD (119 to UD)	62	UD (202 to UD)	0.83 (0.46 to 1.51)	1.39 (0.69 to 2.78)	0.36
		AT	60	UD (144 to UD)	51	UD (155 to UD)	1.22 (0.65 to 2.26)		
		TT	8	UD (280 to UD)	6	274 (274 to UD)	2.36 (0.26 to 21.55)		

Table 7.4 continued.

Gene	SNP	Geno-type	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within subgroup (95% CI) [1]	Hazard ratio for allocation*geno-type interaction (95% CI) [1]	P value for interaction
DHCR7	rs3829251	GG	92	UD (136 to UD)	94	UD (192 to UD)	0.89 (0.55 to 1.45)	1.70 (0.71 to 4.08)	0.24
		AG	28	UD (111 to UD)	30	317 (203 to UD)	1.51 (0.66 to 3.45)		
		AA	1*	-	1*	-	-		
	rs12785878	TT	72	UD (229 to UD)	59	UD (213 to UD)	1.15 (0.65 to 2.04)	0.77 (0.44 to 1.36)	0.37
		GT	37	UD (38 to UD)	47	UD (155 to UD)	0.75 (0.37 to 1.51)		
		GG	13	UD (58 to UD)	18	UD (266 to UD)	0.47 (0.13 to 1.74)		
LRP2	rs3755166	GG	45	UD (144 to UD)	39	UD (274 to UD)	0.82 (0.34 to 1.98)	1.68 (0.91 to 3.12)	0.10
		AG	60	343 (66 to UD)	66	353 (155 to UD)	1.01 (0.60 to 1.69)		
		AA	17	UD (UD to UD)	19	UD (61 to UD)	2.57 (0.60 to 11.03)		
RXRA	rs7861779	GG	74	UD (144 to UD)	80	UD (151 to UD)	1.45 (0.85 to 2.50)	0.57 (0.30 to 1.12)	0.10
		GA	37	UD (58 to UD)	38	UD (220 to UD)	0.64 (0.31 to 1.33)		
		AA	9	UD (218 to UD)	6	UD (221 to UD)	0.38 (0.03 to 5.30)		
	rs9409929	GG	53	UD (126 to UD)	53	UD (151 to UD)	1.04 (0.57 to 1.91)	1.09 (0.60 to 1.98)	0.78
		AG	55	UD (144 to UD)	56	UD (213 to UD)	1.29 (0.67 to 2.50)		
		AA	14	UD (58 to UD)	14	297 (155 to UD)	1.59 (0.48 to 5.22)		
VDR	rs4334089	GG	62	UD (105 to UD)	62	UD (135 to UD)	1.00 (0.57 to 1.75)	1.40 (0.77 to 2.55)	0.27
		AG	42	UD (126 to UD)	48	UD (203 to UD)	1.02 (0.51 to 2.06)		
		AA	18	UD (343 to UD)	13	UD (186 to UD)	2.04 (0.50 to 8.33)		
	rs10783219	AA	54	UD (241 to UD)	59	UD (133 to UD)	1.85 (0.98 to 3.46)	0.55 (0.30 to 1.02)	0.057
		AT	52	UD (136 to UD)	48	UD (297 to UD)	0.60 (0.29 to 1.21)		
		TT	16	105 (35 to UD)	15	328 (151 to UD)	0.71 (0.23 to 2.21)		
	rs4516035	TT	51	UD (77 to UD)	48	UD (186 to UD)	0.93 (0.49 to 1.74)	1.11 (0.63 to 1.92)	0.72
		CT	47	UD (144 to UD)	54	UD (192 to UD)	1.00 (0.52 to 1.93)		
		CC	21	UD (233 to UD)	22	UD (203 to UD)	1.39 (0.49 to 3.97)		
	rs11568820	CC	69	UD (111 to UD)	67	UD (213 to UD)	0.84 (0.48 to 1.47)	1.41 (0.79 to 2.50)	0.24
		CT	35	UD (251 to UD)	36	UD (118 to UD)	1.22 (0.57 to 2.62)		
		TT	15	UD (241 to UD)	17	UD (202 to UD)	1.20 (0.37 to 3.94)		
	rs7976091	CC	69	UD (111 to UD)	69	UD (192 to UD)	0.98 (0.57 to 1.69)	1.23 (0.69 to 2.19)	0.49
		CT	38	UD (251 to UD)	34	UD (128 to UD)	1.15 (0.54 to 2.45)		
		TT	15	UD (241 to UD)	16	UD (202 to UD)	1.07 (0.30 to 3.76)		
	rs2238136	CC	70	UD (141 to UD)	72	UD (128 to UD)	1.03 (0.62 to 1.72)	1.21 (0.64 to 2.30)	0.56
		CT	45	UD (126 to UD)	40	UD (274 to UD)	0.92 (0.39 to 2.16)		
		TT	7	343 (37 to UD)	12	270 (101 to UD)	1.15 (0.19 to 7.05)		
	rs1544410	CC	40	UD (136 to UD)	45	UD (231 to UD)	1.06 (0.50 to 2.29)	1.16 (0.64 to 2.12)	0.63
		CT	59	UD (105 to UD)	57	UD (213 to UD)	0.94 (0.50 to 1.74)		
		TT	22	UD (117 to UD)	20	274 (151 to UD)	1.46 (0.56 to 3.81)		
	rs2228570	GG	51	UD (241 to UD)	51	UD (186 to UD)	1.37 (0.71 to 2.61)	0.85 (0.48 to 1.52)	0.59
		AG	58	UD (91 to UD)	53	UD (202 to UD)	0.71 (0.38 to 1.32)		
		AA	14	UD (269 to UD)	20	UD (135 to UD)	1.62 (0.42 to 6.28)		
	rs2853559	GG	54	UD (105 to UD)	49	UD (158 to UD)	1.35 (0.74 to 2.48)	0.77 (0.42 to 1.43)	0.41
		AG	50	UD (216 to UD)	58	UD (244 to UD)	0.77 (0.39 to 1.52)		
		AA	19	UD (119 to UD)	16	UD (43 to UD)	1.23 (0.38 to 3.99)		
	rs7975232	AA	40	UD (141 to UD)	35	328 (151 to UD)	1.92 (0.92 to 3.99)	0.64 (0.35 to 1.16)	0.14
		AC	57	UD (144 to UD)	54	UD (216 to UD)	0.91 (0.47 to 1.76)		
		CC	20	269 (58 to UD)	31	UD (155 to UD)	0.71 (0.27 to 1.89)		
	rs7970314	AA	68	UD (111 to UD)	70	UD (213 to UD)	0.86 (0.50 to 1.49)	1.46 (0.83 to 2.56)	0.18
		AG	38	UD (251 to UD)	36	UD (118 to UD)	1.26 (0.60 to 2.65)		
		GG	16	UD (241 to UD)	33	364 (186 to UD)	1.45 (0.47 to 4.55)		
	rs731236	AA	45	UD (136 to UD)	50	UD (221 to UD)	0.95 (0.48 to 1.86)	1.23 (0.66 to 2.27)	0.52
		AG	61	UD (216 to UD)	53	UD (192 to UD)	1.15 (0.62 to 2.15)		
		GG	15	UD (111 to UD)	19	353 (158 to UD)	1.61 (0.55 to 4.74)		

[1] *Adjusted for stratification factors i.e. British Thoracic Society treatment step (2/3 vs. 4/5) and inclusion in vs. exclusion from sputum induction sub-study; significant predictors of asthma exacerbation i.e. Baseline asthma control test score (<19 vs. ≥19), reversibility assessment (yes vs. no), and sex; and allocation to study intervention or placebo arm. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for interaction remain significant. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category.*

Abbreviations: SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, UD: Undefined, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor, CUBN: Cubilin.

7.3. Discussion.

In this chapter I have investigated the effect of known variants in common vitamin D pathway genes on the risk of URI or disease exacerbation in patients with asthma. Main effects and interaction analyses did not identify any SNP which directly associate with URI / exacerbation, or modify the effect of vitamin D supplementation in prevention of URI / exacerbation.

The lack of association between vitamin D pathway SNP and risk of URI or asthma exacerbation I report in this adult study population chimes with null reports from two clinical trials of vitamin D supplementation conducted in adult participants (209) (300). If vitamin D supplementation is not an effective intervention for prevention of URI / exacerbation then an argument for the importance of variation in vitamin D related genes becomes harder to make. However, the disruption of genes which control concentrations of the active vitamin D metabolite ($1,25[OH]_2D$) and those which govern vitamin D signalling could affect a change in vitamin D's actions, without being largely dependent on circulating concentrations of 25(OH)D – which is often the sole measure of vitamin D's potential in clinical trials. Interestingly, genetic studies have reported main effect associations between vitamin D pathway SNP and risk of respiratory infections in children (202, 340), while other studies have found vitamin D related SNP to associate with clinical markers of asthma phenotype in children: Pillai et al., and Nabih et al. report an association between rs10735810 (*FokI* mutation in *VDR*) and IgE concentration; FEV_1/FVC spirometric volumes; and Th2 cytokine concentrations (130, 195), and Leung et al. found rs17470271 (*CYP27A1*) to associate with FEV_1 (297). Furthermore, two clinical trials have demonstrated a protective effect of vitamin D supplementation in asthmatic children (280, 299). A possible explanation for the contrasting findings between studies conducted in children and adults is that the developing airway may be more sensitive to the actions of vitamin D than that of adult asthma patients. Another possibility is that limitations of previous clinical trials conducted in adults were responsible for their null reports (e.g. large proportion of moderate asthma patients, and dosing regimens that failed to achieve sustained serum 25-hydroxyvitamin D

concentrations, within the physiological range), and that publication bias has resulted in the lack of null genetic study reports in children.

To further investigate the potential of genetic variation in vitamin D related genes to influence risk of ARI / exacerbations, or influence the effects of vitamin D supplementation in prevention of these outcomes, large clinical trials are needed in severe asthma patients who are genotyped for the SNP I have investigated.

7.3.1. Study strengths.

To my knowledge, this is the first study to comprehensively investigate genetic variation in the vitamin D pathway on risk of upper respiratory infection and disease exacerbation in asthma patients participating in a clinical trial of vitamin D supplementation (209). My analysis utilised genotyping data from a panel of SNP which capture the current body of positive genetic association findings between vitamin D pathway variants and vitamin D metabolite / non-skeletal disease outcomes. Furthermore, my analysis was strengthened by the detailed information I collected on potential confounders of the relationship between vitamin D pathway SNP and risk of URI / asthma exacerbation. 25(OH)D concentrations were measured with the gold standard assay (LC-MS/MS) in a laboratory that participated in the international vitamin D external quality assurance scheme (www.deqas.org/). The study population included patients with mild, moderate and severe disease from both community and hospital settings, studied across all seasons: these features enhance generalisability of our results.

7.3.2. Study limitations.

One limitation of this study was power to detect modest effects of SNP on outcome measures: the clinical trial population was powered to detect the effects of vitamin D supplementation in the entire study population, rather than by SNP genotypes, which may have limited my ability to see a signal most for SNP with the smallest minor allele frequencies and opened the door to the potential for type II error. Another limitation was the way in which URI events were captured: URI were not laboratory-confirmed, however our method of event capture was validated against PCR in a previous study (222). One final possible limitation of this study may have been the use of an intermittent bolus vitamin D dosing regimen, rather than a daily or weekly regimen. Whilst intermittent bolus dosing generally offers better compliance, it has been speculated the sharp boost in vitamin D metabolite concentration which arises from bolus doses may cause dysregulation of the balance between CYP27B1 and CYP24A1 enzymes to result in reduced concentrations of active metabolite at sites of disease (343).

7.4. Conclusions.

Previous genetic studies have found vitamin D pathway SNP to associate with risk of ARI in healthy children, and clinical features of asthma phenotype in children. My analysis found no association between vitamin D pathway SNP and risk of ARI / exacerbation in adult asthma patients, nor did it find these SNP to influence vitamin D supplementation in prevention of ARI / exacerbations, which suggests vitamin D is not an effective intervention for this particular health outcome. However, type II error resulting from a lack of power cannot be discounted as a possible cause of my null results, therefore further investigation into the importance of genetic variation in the vitamin D pathway is called for in larger studies with a wide range of age groups.

8. Genetic variants in the vitamin D pathway and susceptibility to acute respiratory infections in older adults.

In this chapter I will investigate the relationship between single nucleotide polymorphisms in the vitamin D pathway and risk of upper and lower respiratory infection in older adult, sheltered accommodation residents who participated in the ViDiFlu trial (described in Methods, section 2.2.1). This will comprise an investigation of main effects association between SNP and ARI outcomes, and an investigation SNP-mediated effect modification of vitamin D supplementation in prevention of ARI.

8.1. Introduction.

Variation in genes along the vitamin D pathway may be of particular importance to healthy older adult populations, which are at high risk of ARI and vitamin D deficiency. Previous genetic studies have reported main effect associations between rs10735810 (*FokI* mutation) in *VDR* and risk of severe respiratory syncytial virus infection and lower respiratory infection in children (202, 340), but the effect of this variant has not been investigated in older adults, nor have other SNP in vitamin D pathway genes. These genes govern metabolism, transport, and signalling of the micronutrient. At least 55 SNP in 11 vitamin D related genes have shown main effect associations with vitamin D metabolite concentrations and/or a wide range of non-skeletal diseases (reviewed in (335)), and furthermore, the infectious and auto-immune diseases are among the most commonly reported outcomes. An investigation of genetic effect modification of vitamin D supplementation in the prevention of ARI in older adults has not been conducted, despite findings from clinical trials (presented in chapter 3) that show vitamin D is an effective intervention for this health outcome. Clinical studies specifically in older adults are conflicting however: Two cohort studies from Japan report positive associations between serum concentrations of the active vitamin D metabolite,

$1,25(OH)_2D$ and risk of febrile respiratory illness, or hospitalisation due to LRI (261) (262). Whilst clinical trials which have administered a vitamin D intervention for prevention of ARI in older adults have reported null/negative results (267, 273, 344). These results may have arisen due to study limitations e.g. the investigation of ARI as a secondary outcome or by post-hoc analysis, and the use of intermittent bolus dosing, which is of questionable efficacy (345). It is also possible that vitamin D may exert its effects on risk of ARI independent of serum 25(OH)D concentration. Or, it may be that previous positive observational findings have occurred due to uncontrolled confounding.

To my knowledge a comprehensive investigation into the influence of genetic variation in the vitamin D pathway on risk of ARI in older adults has not been conducted. I therefore carried out a main effect analysis between 35 SNP in 11 vitamin D related genes and risk of ARI, and an interaction analysis between these SNP and allocation to vitamin D supplementation or placebo, for the prevention of ARI in older adults.

8.2. Results.

8.2.1. Main effects analysis: does vitamin D pathway genotype influence risk of ARI, independent of vitamin D supplementation?

Results of main effects analyses are presented in Table 8.1 and Table 8.2. Multivariable Cox regression analysis (described in Methods, section 2.4.2) identified the following SNP genotypes to associate with risk of URI: referent to CC genotype rs2248137 (*CYP24A1*), CG genotype associates with a 19% higher risk, and GG genotype associates with a 80% higher risk (P value for trend = 0.026); referent to AA genotype for rs4646536 (*CYP27B1*), AG genotype associates with a 22% lower risk, and GG genotype associates with a 65% lower risk (P value for trend = 0.005).

Multivariable Cox regression analysis also identified 1 SNP to associate with risk of LRI: referent to AA genotype for rs4646536 (*CYP27B1*), AG genotype associates with a 18% lower risk, and GG genotype associates with a 69% lower risk (P value for trend = 0.007).

After correction for multiple comparisons testing (Bejamini & Hochberg method with a false discovery rate of 5%) none of the observed main effect associations between SNP and risk of URI or LRI remained significant.

Table 8.1. Main effects: Time to first URI.

Gene	SNP	Geno-type	N	Days at risk (IQR)	Univariable Hazard Ratio (95% CI)	P value	Multivariable Hazard Ratio (95% CI) [1]	P value
CUBN	rs3740165	TT	207	300 (87 to UD)	referent	0.52	referent	0.79
		TC	20	UD (83 to UD)	0.92 (0.46 to 1.81)		1.07 (0.54 to 2.14)	
		CC	1*	-	-		-	
CYP24A1	rs2762939	GG	104	227 (85 to UD)	referent	0.17	referent	0.14
		CG	95	284 (80 to UD)	0.89 (0.61 to 1.29)		0.81 (0.55 to 1.19)	
		CC	32	UD (149 to UD)	0.64 (0.34 to 1.19)		0.67 (0.35 to 1.26)	
	rs2248137	CC	75	UD (75 to UD)	referent	0.10	referent	0.026
		CG	104	286 (82 to UD)	1.19 (0.78 to 1.82)		1.19 (0.78 to 1.82)	
		GG	54	210 (58 to UD)	1.50 (0.93 to 2.43)		1.80 (1.10 to 2.94)	
	rs2762934	GG	156	301 (89 to UD)	referent	0.53	referent	0.47
		AG	61	276 (75 to UD)	1.12 (0.74 to 1.69)		1.12 (0.02 to 1.34)	
		AA	10*	-	-		-	
	rs6013897	TT	141	286 (88 to UD)	referent	0.72	referent	0.39
		AT	79	301 (87 to UD)	1.01 (0.69 to 1.49)		1.14 (0.77 to 1.70)	
		AA	12	203 (41 to UD)	1.26 (0.55 to 2.89)		1.33 (0.57 to 3.08)	
CYP27A1	rs17470271	AA	92	339 (107 to UD)	referent	0.85	referent	0.89
		AT	102	227 (60 to UD)	1.35 (0.92 to 2.00)		1.29 (0.87 to 1.92)	
		TT	38	UD (126 to UD)	0.91 (0.53 to 1.58)		0.85 (0.48 to 1.49)	
CYP27B1	rs4646537	TT	202	301 (87 to UD)	referent	0.78	referent	0.90
		GT	24	220 (89 to UD)	1.09 (0.60 to 1.98)		1.04 (0.56 to 1.94)	
		GG	0*	-	-		-	
	rs4646536	AA	115	205 (63 to UD)	referent	0.004	referent	0.005
		AG	90	300 (130 to UD)	0.75 (0.51 to 1.09)		0.78 (0.53 to 1.15)	
		GG	30	UD (203 to UD)	0.36 (0.17 to 0.76)		0.35 (0.17 to 0.74)	
CYP2R1	rs10500804	TT	105	UD (117 to UD)	referent	0.11	referent	0.18
		GT	99	265 (83 to UD)	1.28 (0.87 to 1.88)		1.26 (0.85 to 1.86)	
		GG	32	141 (75 to UD)	1.46 (0.86 to 2.49)		1.37 (0.80 to 2.34)	
	rs2060793	GG	77	217 (76 to UD)	referent	0.23	referent	0.33
		AG	113	286 (96 to UD)	0.79 (0.53 to 1.18)		0.79 (0.47 to 1.32)	
		AA	45	329 (100 to UD)	0.75 (0.45 to 1.26)		0.84 (0.56 to 1.26)	
	rs10766197	GG	94	UD (118 to UD)	referent	0.14	referent	0.19
		AG	100	284 (83 to UD)	1.20 (0.80 to 1.80)		1.15 (0.77 to 1.73)	
		AA	32	141 (82 to UD)	1.50 (0.87 to 2.58)		1.45 (0.84 to 2.50)	
CYP3A4	rs2740574	AA	181	280 (83 to UD)	referent	0.39	referent	0.47
		AG	29	339 (125 to UD)	0.84 (0.48 to 1.48)		0.81 (0.46 to 1.46)	
		GG	24	UD (154 to UD)	0.79 (0.41 to 1.51)		0.83 (0.42 to 1.64)	
DBP	rs7041	CC	60	304 (75 to UD)	referent	0.48	referent	0.43
		AC	105	300 (109 to UD)	0.94 (0.60 to 1.46)		0.96 (0.62 to 1.51)	
		AA	67	265 (75 to UD)	1.19 (0.73 to 1.92)		1.22 (0.74 to 2.00)	
	rs4588	GG	137	339 (87 to UD)	referent	0.25	referent	0.41
		GT	86	234 (85 to UD)	1.28 (0.89 to 1.86)		1.23 (0.85 to 1.79)	
		TT	13	159 (63 to UD)	1.17 (0.51 to 2.69)		1.05 (0.45 to 2.49)	
	rs12512631	TT	112	265 (75 to UD)	referent	0.19	referent	0.17
		CT	100	304 (96 to UD)	0.85 (0.58 to 1.24)		0.88 (0.60 to 1.28)	
		CC	23	UD (115 to UD)	0.67 (0.34 to 1.30)		0.63 (0.32 to 1.23)	
	rs2070741	TT	188	297 (88 to UD)	referent	0.68	referent	0.90
		TG	36	305 (59 to UD)	0.97 (0.58 to 1.62)		1.15 (0.68 to 1.95)	
		GG	3*	-	-		0.60 (0.08 to 4.35)	
	rs2298849	AA	132	234 (82 to UD)	referent	0.38	referent	0.88
		AG	75	UD (109 to UD)	0.73 (0.48 to 1.11)		0.81 (0.53 to 1.24)	
		GG	25	305 (52 to UD)	0.95 (0.53 to 1.71)		1.16 (0.63 to 2.13)	
	rs16846876	AA	116	339 (89 to UD)	referent	0.22	referent	0.24
		AT	103	234 (83 to UD)	1.23 (0.85 to 1.78)		1.25 (0.85 to 1.84)	
		TT	18	159 (86 to UD)	1.35 (0.69 to 2.64)		1.32 (0.66 to 2.63)	
DHCR7	rs3829251	GG	165	266 (87 to UD)	referent	0.89	referent	0.91

Table 8.1 continued.

Gene	SNP	Geno-type	N	Days at risk (IQR)	Univariable Hazard Ratio (95% CI)	P value	Multivariable Hazard Ratio (95% CI) [1]	P value
		AG	55	UD (107 to UD)	0.86 (0.55 to 1.33)		0.86 (0.55 to 1.34)	
		AA	8	117 (12 to UD)	1.36 (0.55 to 3.35)		1.37 (0.55 to 3.38)	
	rs12785878	TT	115	266 (86 to UD)	referent	0.36	referent	0.28
		GT	82	UD (126 to UD)	0.77 (0.51 to 1.16)		0.80 (0.53 to 1.21)	
		GG	38	132 (39 to UD)	1.51 (0.94 to 2.42)		1.63 (0.98 to 2.71)	
LRP2	rs3755166	GG	97	280 (88 to UD)	referent	0.43	referent	0.43
		AG	112	304 (80 to UD)	0.89 (0.61 to 1.30)		0.92 (0.63 to 1.35)	
		AA	28	UD (83 to UD)	0.81 (0.44 to 1.48)		0.78 (0.43 to 1.45)	
RXRA	rs7861779	GG	139	286 (83 to UD)	referent	0.89	referent	0.92
		GA	61	300 (100 to UD)	1.06 (0.70 to 1.61)		1.04 (0.68 to 1.60)	
		AA	24	363 (76 to UD)	1.00 (0.53 to 1.89)		1.00 (0.52 to 1.92)	
	rs9409929	GG	123	304 (100 to UD)	referent	0.88	referent	0.59
		AG	88	262 (59 to UD)	1.20 (0.83 to 1.75)		1.07 (0.73 to 1.56)	
		AA	23	UD (89 to UD)	0.85 (0.44 to 1.66)		0.72 (0.36 to 1.43)	
VDR	rs4334089	GG	116	234 (63 to UD)	referent	0.36	referent	0.54
		AG	79	UD (129 to UD)	0.74 (0.49 to 1.11)		0.81 (0.54 to 1.24)	
		AA	37	339 (59 to UD)	0.89 (0.53 to 1.49)		0.92 (0.54 to 1.57)	
	rs10783219	AA	109	301 (83 to UD)	referent	0.57	referent	0.93
		AT	87	286 (100 to UD)	0.99 (0.67 to 1.48)		0.94 (0.63 to 1.41)	
		TT	34	266 (85 to UD)	1.21 (0.72 to 2.04)		1.07 (0.62 to 1.82)	
	rs4516035	TT	107	266 (75 to UD)	referent	0.25	referent	0.25
		CT	88	284 (89 to UD)	0.98 (0.66 to 1.45)		1.01 (0.68 to 1.51)	
		CC	31	UD (159 to UD)	0.65 (0.35 to 1.21)		0.62 (0.32 to 1.18)	
	rs11568820	CC	135	284 (87 to UD)	referent	0.93	referent	0.81
		CT	59	328 (129 to UD)	0.81 (0.54 to 1.30)		0.89 (0.57 to 1.38)	
		TT	40	297 (75 to UD)	1.05 (0.65 to 1.71)		1.14 (0.69 to 1.88)	
	rs7976091	CC	135	286 (88 to UD)	referent	0.88	referent	0.72
		CT	57	328 (129 to UD)	0.85 (0.55 to 1.33)		0.89 (0.57 to 1.39)	
		TT	39	297 (75 to UD)	1.12 (0.69 to 1.83)		1.18 (0.71 to 1.95)	
	rs2238136	CC	146	301 (88 to UD)	referent	0.55	referent	0.65
		CT	78	329 (100 to UD)	0.95 (0.64 to 1.40)		0.94 (0.63 to 1.40)	
		TT	13	89 (30 to UD)	1.65 (0.79 to 3.42)		1.50 (0.72 to 3.14)	
	rs1544410	CC	84	231 (88 to UD)	referent	0.19	referent	0.34
		CT	110	284 (82 to UD)	1.00 (0.67 to 1.47)		1.04 (0.70 to 1.53)	
		TT	33	UD (118 to UD)	0.58 (0.30 to 1.12)		0.64 (0.33 to 1.25)	
	rs2228570	GG	106	304 (83 to UD)	referent	0.65	referent	0.41
		AG	99	284 (107 to UD)	1.10 (0.75 to 1.62)		1.16 (0.78 to 1.71)	
		AA	27	280 (82 to UD)	1.09 (0.61 to 1.97)		1.22 (0.67 to 2.22)	
	rs2853559	GG	98	266 (85 to UD)	referent	0.59	referent	0.56
		AG	111	328 (88 to UD)	0.92 (0.63 to 1.34)		0.89 (0.60 to 1.30)	
		AA	28	301 (75 to UD)	0.87 (0.48 to 1.57)		0.88 (0.49 to 1.61)	
	rs7975232	AA	71	UD (107 to UD)	referent	0.20	referent	0.29
		AC	121	266 (80 to UD)	1.33 (0.87 to 2.03)		1.26 (0.82 to 1.93)	
		CC	40	220 (89 to UD)	1.38 (0.80 to 2.39)		1.31 (0.76 to 2.28)	
	rs7970314	AA	126	286 (88 to UD)	referent	0.96	referent	0.62
		AG	63	280 (83 to UD)	0.99 (0.65 to 1.52)		1.06 (0.69 to 1.63)	
		GG	48	305 (75 to UD)	1.02 (0.64 to 1.62)		1.23 (0.69 to 1.84)	
	rs731236	AA	97	329 (96 to UD)	referent	0.45	referent	0.57
		AG	106	265 (80 to UD)	1.17 (0.80 to 1.70)		1.19 (0.82 to 1.75)	
		GG	31	UD (107 to UD)	0.61 (0.31 to 1.21)		0.64 (0.32 to 1.27)	

[1] *Adjusted for study group (resident vs. carer); minimisation variables (level of care, size of scheme and season of randomisation); a significant predictor of upper respiratory infection i.e. baseline vitamin D status (<50 nmol/L vs. ≥50 nmol/L); and allocation to study intervention or placebo arm. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for interaction remain significant. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category.*

Abbreviations: SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, UD: Undefined, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor.

Table 8.2. Main effects: Time to first LRI.

Gene	SNP	Geno-type	N	Days at risk (IQR)	Univariable Hazard Ratio (95% CI)	P value	Multivariable Hazard Ratio [1] (95% CI)	P value
CUBN	rs3740165	TT	207	UD (127 to UD)	referent	0.92	referent	0.62
		TC	20	UD (86 to UD)	1.13 (0.57 to 2.23)		0.93 (0.43 to 1.99)	
		CC	1*	-	-		-	
CYP24A1	rs2762939	GG	104	UD (118 to UD)	referent	0.71	referent	0.89
		CG	95	361 (111 to UD)	1.24 (0.82 to 1.86)		1.14 (0.75 to 1.74)	
		CC	32	UD (151 to UD)	0.97 (0.51 to 1.84)		0.89 (0.41 to 1.93)	
	rs2248137	CC	75	UD (141 to UD)	referent	0.66	referent	0.29
		CG	104	UD (111 to UD)	1.14 (0.73 to 1.79)		1.21 (0.76 to 1.91)	
		GG	54	363 (125 to UD)	1.11 (0.66 to 1.88)		1.35 (0.75 to 2.44)	
	rs2762934	GG	156	UD (136 to UD)	referent	0.66	referent	0.63
		AG	61	284 (111 to UD)	1.40 (0.93 to 2.13)		1.39 (0.91 to 2.12)	
		AA	10*	-	-		-	
	rs6013897	TT	141	UD (117 to UD)	referent	0.16	referent	0.24
		AT	79	363 (126 to UD)	1.14 (0.75 to 1.72)		1.09 (0.71 to 1.68)	
		AA	12	203 (40 to UD)	1.96 (0.89 to 4.29)		1.88 (0.84 to 4.17)	
CYP27A1	rs17470271	AA	92	UD (109 to UD)	referent	0.44	referent	0.65
		AT	102	363 (88 to UD)	0.99 (0.65 to 1.50)		1.08 (0.68 to 1.71)	
		TT	38	UD (224 to UD)	0.77 (0.43 to 1.37)		0.83 (0.45 to 1.53)	
CYP27B1	rs4646537	TT	202	363 (117 to UD)	referent	0.10	referent	0.12
		GT	24	UD (306 to UD)	0.50 (0.22 to 1.14)		0.50 (0.21 to 1.18)	
		GG	0*	-	-		-	
	rs4646536	AA	115	361 (89 to UD)	referent	0.066	referent	0.007
		AG	90	UD (141 to UD)	0.86 (0.57 to 1.29)		0.82 (0.54 to 1.25)	
		GG	30	UD (203 to UD)	0.49 (0.23 to 1.03)		0.31 (0.14 to 0.70)	
CYP2R1	rs10500804	TT	105	UD (137 to UD)	referent	0.51	referent	0.35
		GT	99	362 (109 to UD)	1.25 (0.83 to 1.89)		1.28 (0.82 to 2.00)	
		GG	32	UD (119 to UD)	1.11 (0.62 to 2.00)		1.25 (0.67 to 2.33)	
	rs2060793	GG	77	361 (125 to UD)	referent	0.71	referent	0.94
		AG	113	UD (115 to UD)	0.89 (0.58 to 1.37)		0.91 (0.59 to 1.41)	
		AA	45	UD (117 to UD)	0.92 (0.54 to 1.59)		1.06 (0.61 to 1.85)	
	rs10766197	GG	94	UD (125 to UD)	referent	0.84	referent	0.64
		AG	100	363 (88 to UD)	1.11 (0.73 to 1.69)		1.10 (0.69 to 1.74)	
		AA	32	UD (119 to UD)	1.01 (0.56 to 1.83)		1.15 (0.61 to 2.16)	
CYP3A4	rs2740574	AA	181	UD (115 to UD)	referent	0.96	referent	0.22
		AG	29	UD (125 to UD)	1.04 (0.58 to 1.86)		0.65 (0.31 to 1.37)	
		GG	24	UD (154 to UD)	0.96 (0.50 to 1.86)		0.53 (0.17 to 1.68)	
DBP	rs7041	CC	60	UD (115 to UD)	referent	0.66	referent	0.93
		AC	105	UD (137 to UD)	0.86 (0.53 to 1.38)		0.76 (0.47 to 1.25)	
		AA	67	339 (105 to UD)	1.12 (0.67 to 1.86)		1.07 (0.59 to 1.93)	
	rs4588	GG	137	UD (118 to UD)	referent	0.96	referent	0.86
		GT	86	UD (117 to UD)	1.03 (0.69 to 1.53)		0.99 (0.65 to 1.49)	
		TT	13	UD (109 to UD)	0.98 (0.39 to 2.43)		0.89 (0.34 to 2.38)	
	rs12512631	TT	112	346 (109 to UD)	referent	0.18	referent	0.38
		CT	100	UD (118 to UD)	0.87 (0.58 to 1.29)		0.91 (0.60 to 1.37)	
		CC	23	UD (138 to UD)	0.61 (0.29 to 1.28)		0.72 (0.34 to 1.53)	
	rs2070741	TT	188	UD (118 to UD)	referent	0.74	referent	0.70
		TG	36	UD (137 to UD)	0.95 (0.55 to 1.65)		0.92 (0.52 to 1.64)	
		GG	3*	-	-		-	
	rs2298849	AA	132	UD (115 to UD)	referent	0.40	referent	0.29
		AG	75	UD (126 to UD)	0.90 (0.58 to 1.39)		1.00 (0.64 to 1.58)	
		GG	25	273 (56 to UD)	1.50 (0.85 to 2.65)		1.50 (0.83 to 2.72)	
	rs16846876	AA	116	UD (136 to UD)	referent	0.64	referent	0.60
		AT	103	363 (109 to UD)	1.19 (0.80 to 1.77)		1.20 (0.79 to 1.83)	
		TT	18	UD (88 to UD)	0.97 (0.44 to 2.13)		1.01 (0.44 to 2.32)	
DHCR7	rs3829251	GG	165	UD (114 to UD)	referent	0.98	referent	0.86

Table 8.2 continued.

Gene	SNP	Geno-type	N	Days at risk (IQR)	Univariable Hazard Ratio (95% CI)	P value	Multivariable Hazard Ratio [1] (95% CI)	P value
		AG	55	UD (148 to UD)	0.83 (0.52 to 1.32)		0.81 (0.51 to 1.31)	
		AA	8	117 (10 to UD)	1.67 (0.67 to 4.12)		1.46 (0.57 to 3.74)	
	rs12785878	TT	115	UD (138 to UD)	referent	0.036	referent	0.18
		GT	82	363 (137 to UD)	1.20 (0.78 to 1.84)		1.17 (0.74 to 1.84)	
		GG	38	232 (50 to UD)	1.79 (1.07 to 3.00)		1.60 (0.83 to 3.10)	
LRP2	rs3755166	GG	97	337 (132 to UD)	referent	0.11	referent	0.081
		AG	112	UD (114 to UD)	0.92 (0.62 to 1.37)		0.86 (0.57 to 1.30)	
		AA	28	UD (273 to UD)	0.50 (0.24 to 1.06)		0.49 (0.23 to 1.06)	
RXRA	rs7861779	GG	139	UD (117 to UD)	referent	0.41	referent	0.48
		GA	61	306 (126 to UD)	1.31 (0.85 to 2.01)		1.32 (0.85 to 2.06)	
		AA	24	UD (105 to UD)	1.10 (0.56 to 2.16)		1.01 (0.44 to 2.32)	
	rs9409929	GG	123	UD (127 to UD)	referent	0.55	referent	0.34
		AG	88	363 (88 to UD)	1.15 (0.76 to 1.72)		1.26 (0.82 to 1.93)	
		AA	23	UD (119 to UD)	1.13 (0.59 to 2.16)		1.23 (0.63 to 2.40)	
VDR	rs4334089	GG	116	UD (115 to UD)	referent	0.75	referent	0.82
		AG	79	UD (138 to UD)	0.88 (0.57 to 1.36)		0.92 (0.58 to 1.43)	
		AA	37	339 (60 to UD)	1.20 (0.70 to 2.04)		1.19 (0.62 to 2.29)	
	rs10783219	AA	109	346 (86 to UD)	referent	0.42	referent	0.15
		AT	87	UD (136 to UD)	0.87 (0.58 to 1.32)		0.76 (0.48 to 1.20)	
		TT	34	UD (109 to UD)	0.82 (0.45 to 1.47)		0.65 (0.34 to 1.24)	
	rs4516035	TT	107	UD (111 to UD)	referent	0.57	referent	0.81
		CT	88	363 (132 to UD)	0.97 (0.64 to 1.48)		1.21 (0.74 to 1.98)	
		CC	31	UD (137 to UD)	0.81 (0.43 to 1.52)		1.00 (0.50 to 2.01)	
	rs11568820	CC	135	UD (127 to UD)	referent	0.45	referent	0.35
		CT	59	UD (109 to UD)	1.12 (0.71 to 1.77)		1.12 (0.70 to 1.77)	
		TT	40	339 (105 to UD)	1.20 (0.71 to 2.01)		1.52 (0.67 to 3.45)	
	rs7976091	CC	135	UD (132 to UD)	referent	0.36	referent	0.20
		CT	57	UD (109 to UD)	1.14 (0.72 to 1.81)		1.12 (0.70 to 1.79)	
		TT	39	339 (60 to UD)	1.25 (0.75 to 2.11)		1.91 (0.83 to 4.36)	
	rs2238136	CC	146	UD (118 to UD)	referent	0.41	referent	0.35
		CT	78	UD (132 to UD)	0.94 (0.62 to 1.40)		0.89 (0.58 to 1.35)	
		TT	13	UD (89 to UD)	0.62 (0.23 to 1.70)		0.63 (0.23 to 1.76)	
	rs1544410	CC	84	UD (138 to UD)	referent	0.66	referent	0.53
		CT	110	313 (88 to UD)	1.39 (0.91 to 2.13)		1.49 (0.97 to 2.30)	
		TT	33	UD (137 to UD)	0.95 (0.49 to 1.84)		0.98 (0.50 to 1.93)	
	rs2228570	GG	106	UD (109 to UD)	referent	0.64	referent	0.89
		AG	99	UD (170 to UD)	0.86 (0.57 to 1.30)		0.90 (0.59 to 1.38)	
		AA	27	UD (89 to UD)	0.94 (0.50 to 1.77)		1.04 (0.54 to 1.98)	
	rs2853559	GG	98	302 (105 to UD)	referent	0.077	referent	0.14
		AG	111	UD (132 to UD)	0.66 (0.44 to 0.99)		0.65 (0.42 to 1.00)	
		AA	28	UD (118 to UD)	0.70 (0.36 to 1.34)		0.77 (0.39 to 1.53)	
	rs7975232	AA	71	UD (132 to UD)	referent	0.44	referent	0.51
		AC	121	313 (88 to UD)	1.40 (0.89 to 2.20)		1.36 (0.86 to 2.15)	
		CC	40	UD (126 to UD)	1.18 (0.65 to 2.14)		1.15 (0.63 to 2.12)	
	rs7970314	AA	126	UD (136 to UD)	referent	0.21	referent	0.26
		AG	63	346 (109 to UD)	1.30 (0.83 to 2.03)		1.24 (0.79 to 1.96)	
		GG	48	339 (60 to UD)	1.31 (0.80 to 2.14)		1.42 (0.65 to 3.13)	
	rs731236	AA	97	UD (136 to UD)	referent	0.96	referent	0.97
		AG	106	302 (88 to UD)	1.35 (0.90 to 2.02)		1.32 (0.87 to 2.01)	
		GG	31	UD (183 to UD)	0.78 (0.39 to 1.55)		0.79 (0.39 to 1.60)	

[1] *Adjusted for study group (resident vs. carer); minimisation variables (level of care, size of scheme and season of randomisation); a significant predictor of lower respiratory infection i.e. ethnicity; and allocation to study intervention or placebo arm. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for interaction remain significant.* ******* *Genotype could not be analysed due to <5 participants and/or too few events within this sub category.*

Abbreviations: SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, UD: Undefined, CI: Confidence interval, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor.

8.2.2. *Interaction analysis: does genotype influence effect of vitamin D supplementation in prevention of ARI?*

Results of interaction analyses testing for effect modification are presented in Table 8.3 and Table 8.4. Multivariable Cox regression analysis (described in Methods, section 2.4.3) identified 2 SNP which modify the effect of vitamin D supplementation on risk of URI. Vitamin D supplementation conferred increased risk of URI to individuals with AA genotype for rs17470271 in *CYP27A1* (aHR 2.47; 95% CI 1.29 to 4.73), but had no effect of those with AT genotype (aHR 1.28; 95% CI 0.73 to 2.24), or TT genotype (aHR 0.72; 95% CI 0.27 to 1.95 - aRHR 0.55; 95% CI 0.33 to 0.92; P value for interaction = 0.022). Vitamin D supplementation offered protection for URI to individuals with AA genotype for rs7975232 in *VDR* (aHR 0.85; 95% CI 0.40 to 1.79), and increased risk of URI to those with AC genotype (aHR 1.84; 95% CI 1.08 to 3.14), and CC genotype (aHR 3.21; 95% CI 1.03 to 10.02 - aRHR 1.80; 95% CI 1.01 to 3.20; P value for interaction = 0.046).

Multivariable Cox regression analysis also identified 1 SNP which modifies the effect of vitamin D supplementation on risk of LRI. Vitamin D supplementation conferred increased risk of LRI to individuals with GG genotype for rs10766197 in *CYP2R1* (aHR 3.18; 95% CI 1.50 to 6.74), but had no effect on those with AG genotype (aHR 0.78; 95% CI 0.43 to 1.43), or AA genotype (aHR 0.91; 95% CI 0.32 to 2.55 – aRHR 0.51; 95% CI 0.30 to 0.92; P value for interaction = 0.025).

After correction for multiple comparisons testing (Bejamini & Hochberg method with a false discovery rate of 5%) none of the observed associations of modified effect of vitamin D supplementation remained significant.

Table 8.3. Interaction analysis: Time to first URI.

Gene	SNP	Geno-type	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within subgroup (95% CI)	Hazard ratio for allocation*geno-type interaction (95% CI)	P value for interaction [1]
CUBN	rs3740165	TT	85	UD (132 to UD)	122	231 (75 to UD)	1.70 (1.12 to 2.57)	0.61 (0.15 to 2.47)	0.49
		TC	12	297 (83 to UD)	8	UD (33 to UD)	2.00 (0.36 to 11.12)		
		CC	1*	-	0	-	-		
CYP24A1	rs2762939	GG	45	286 (100 to UD)	59	220 (75 to UD)	1.31 (0.77 to 2.23)	1.49 (0.85 to 2.62)	0.17
		CG	38	UD (83 to UD)	57	265 (60 to UD)	1.38 (0.74 to 2.57)		
		CC	19	UD (339 to UD)	13	149 (58 to UD)	4.90 (1.09 to 22.04)		
	rs2248137	CC	29	UD (134 to UD)	46	301 (126 to UD)	1.53 (0.74 to 3.20)	1.05 (0.63 to 1.74)	0.86
		CG	44	UD (132 to UD)	60	220 (59 to UD)	1.59 (0.89 to 2.84)		
		GG	28	297 (100 to UD)	26	149 (47 to UD)	2.32 (1.05 to 5.12)		
	rs2762934	GG	67	UD (100 to UD)	89	265 (88 to UD)	1.41 (0.89 to 2.24)	1.77 (0.83 to 3.78)	0.14
		AG	26	UD (132 to UD)	35	149 (41 to UD)	1.52 (0.72 to 3.23)		
		AA	5*	-	5*	-	-		
	rs6013897	TT	58	286 (83 to UD)	83	305 (89 to UD)	1.07 (0.66 to 1.73)	2.42 (1.20 to 4.86)	0.013
		AT	39	UD (171 to UD)	40	210 (48 to UD)	2.17 (1.11 to 4.26)		
		AA	4*	-	8	58 (24 to 203)	-		
CYP27A1	rs17470271	AA	42	UD (176 to UD)	50	172 (50 to UD)	2.47 (1.29 to 4.73)	0.55 (0.33 to 0.92)	0.022
		AT	43	223 (63 to UD)	58	227 (58 to UD)	1.28 (0.73 to 2.24)		
		TT	14	300 (159 to UD)	24	UD (117 to UD)	0.72 (0.27 to 1.95)		
CYP27B1	rs4646537	TT	88	UD (132 to UD)	114	234 (75 to UD)	1.68 (1.11 to 2.54)	1.10 (0.31 to 3.98)	0.88
		GT	9	UD (154 to UD)	15	220 (89 to UD)	0.99 (0.15 to 6.30)		
		GG	0	-	0	-	-		
	rs4646536	AA	44	286 (76 to UD)	71	173 (59 to UD)	1.41 (0.83 to 2.40)	1.16 (0.65 to 2.08)	0.62
		AG	46	UD (176 to UD)	44	265 (109 to UD)	1.57 (0.87 to 2.85)		
		GG	11	UD (UD to UD)	19	UD (75 to UD)	2.24 (0.42 to 11.78)		
CYP2R1	rs10500804	TT	49	UD (132 to UD)	56	304 (75 to UD)	1.34 (0.73 to 2.47)	0.78 (0.46 to 1.33)	0.36
		GT	40	UD (83 to UD)	59	220 (58 to UD)	1.71 (0.95 to 3.08)		
		GG	12	107 (63 to UD)	20	141 (75 to UD)	0.76 (0.28 to 2.07)		
	rs2060793	GG	34	297 (83 to UD)	43	164 (60 to UD)	1.43 (0.76 to 2.69)	1.14 (0.67 to 1.94)	0.64
		AG	49	UD (134 to UD)	64	227 (80 to UD)	1.67 (0.94 to 2.96)		
		AA	19	UD (132 to UD)	26	304 (50 to UD)	1.56 (0.61 to 4.00)		
	rs10766197	GG	46	UD (154 to UD)	48	262 (75 to UD)	1.78 (0.92 to 3.42)	0.67 (0.39 to 1.15)	0.15
		AG	39	300 (100 to UD)	61	234 (75 to UD)	1.41 (0.79 to 2.54)		
		AA	16	107 (63 to UD)	16	141 (88 to UD)	1.00 (0.39 to 2.58)		
CYP3A4	rs2740574	AA	72	300 (83 to UD)	109	227 (75 to UD)	1.36 (0.89 to 2.07)	1.36 (0.74 to 2.51)	0.32
		AG	13	UD (339 to UD)	16	173 (55 to UD)	2.38 (0.70 to 8.06)		
		GG	16	UD (176 to UD)	8	265 (42 to UD)	1.93 (0.43 to 8.72)		
DBP	rs7041	CC	24	UD (125 to UD)	36	173 (42 to UD)	0.95 (0.45 to 2.03)	1.42 (0.84 to 2.38)	0.19
		AC	44	UD (129 to UD)	61	247 (107 to UD)	1.32 (0.74 to 2.35)		
		AA	31	284 (59 to UD)	36	304 (75 to UD)	2.17 (1.05 to 4.48)		
	rs4588	GG	61	UD (118 to UD)	76	304 (60 to UD)	1.40 (0.84 to 2.32)	1.25 (0.67 to 2.33)	0.47
		GT	35	UD (107 to UD)	51	227 (80 to UD)	1.59 (0.86 to 2.92)		
		TT	5	UD (159 to UD)	8	126 (55 to UD)	4.57 (0.31 to 66.68)		
	rs12512631	TT	45	UD (132 to UD)	67	149 (55 to UD)	2.25 (1.29 to 3.93)	0.62 (0.35 to 1.10)	0.10
		CT	46	UD (100 to UD)	54	304 (96 to UD)	1.16 (0.64 to 2.10)		
		CC	9	UD (211 to UD)	14	UD (115 to UD)	0.45 (0.09 to 2.39)		
	rs2070741	TT	80	UD (125 to UD)	108	247 (75 to UD)	1.48 (0.97 to 2.27)	0.55 (0.20 to 1.52)	0.25
		TG	17	UD (118 to UD)	19	205 (41 to UD)	3.17 (1.04 to 9.62)		
		GG	1*	-	2*	-	-		
	rs2298849	AA	51	280 (83 to UD)	81	231 (60 to UD)	1.15 (0.71 to 1.86)	1.74 (0.96 to 3.16)	0.068
		AG	39	UD (154 to UD)	36	118 (80 to UD)	2.78 (1.31 to 5.93)		
		GG	11	UD (76 to UD)	14	112 (41 to UD)	2.53 (0.62 to 10.29)		
	rs16846876	AA	49	UD (100 to UD)	67	329 (87 to UD)	1.13 (0.65 to 1.95)	1.66 (0.90 to 3.04)	0.10
		AT	46	UD (132 (to UD)	57	203 (55 to UD)	2.16 (1.20 to 3.87)		
		TT	7	UD (129 to UD)	11	88 (80 to UD)	1.71 (0.33 to 8.89)		

Table 8.3 continued.

Gene	SNP	Geno-type	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within subgroup (95% CI)	Hazard ratio for allocation*geno-type interaction (95% CI)	P value for interaction [1]
DHCR7	rs3829251	GG	68	UD (134 to UD)	97	210 (60 to UD)	1.79 (1.12 to 2.86)	0.74 (0.35 to 1.55)	0.42
		AG	27	UD (132 to UD)	28	305 (55 to UD)	1.52 (0.67 to 3.46)		
		AA	3*	-	5	231 (117 to UD)	-		
	rs12785878	TT	41	300 (100 to UD)	74	247 (85 to UD)	1.25 (0.73 to 2.15)	1.33 (0.78 to 2.29)	0.30
		GT	40	UD (159 to UD)	42	265 (88 to UD)	1.70 (0.87 to 3.33)		
		GG	20	176 (39 to UD)	18	107 (24 to 305)	1.88 (0.73 to 4.83)		
LRP2	rs3755166	GG	46	297 (100 to UD)	51	266 (88 to UD)	1.18 (0.68 to 2.04)	1.12 (0.65 to 1.96)	0.68
		AG	42	UD (216 to UD)	70	170 (47 to UD)	2.28 (1.23 to 4.21)		
		AA	14	284 (83 to UD)	14	UD (126 to UD)	0.96 (0.27 to 3.38)		
RXRA	rs7861779	GG	55	UD (132 to UD)	84	220 (60 to UD)	1.77 (1.04 to 2.99)	1.03 (0.57 to 1.86)	0.94
		GA	29	297 (118 to UD)	32	304 (87 to UD)	1.22 (0.59 to 2.53)		
		AA	11	UD (UD to UD)	13	173 (75 to UD)	3.83 (0.59 to 24.78)		
	rs9409929	GG	59	UD (118 to UD)	64	220 (85 to UD)	1.74 (1.01 to 2.98)	1.04 (0.60 to 1.83)	0.88
		AG	32	UD (59 to UD)	56	227 (55 to UD)	1.48 (0.81 to 2.72)		
		AA	10	UD (154 to UD)	13	UD (89 to UD)	1.04 (0.24 to 4.47)		
VDR	rs4334089	GG	41	UD (63 to UD)	75	220 (80 to UD)	1.68 (0.97 to 2.92)	1.03 (0.60 to 1.75)	0.92
		AG	40	UD (134 to UD)	39	UD (75 to UD)	1.11 (0.56 to 2.20)		
		AA	20	UD (76 to UD)	17	173 (59 to UD)	1.67 (0.56 to 4.92)		
	rs10783219	AA	55	UD (125 to UD)	54	276 (59 to UD)	1.55 (0.87 to 2.76)	1.08 (0.60 to 1.92)	0.80
		AT	36	UD (107 to UD)	51	205 (87 to UD)	2.14 (1.12 to 4.09)		
		TT	9	300 (42 to UD)	25	231 (88 to UD)	1.01 (0.30 to 3.41)		
	rs4516035	TT	45	UD (154 to UD)	62	170 (48 to UD)	2.30 (1.26 to 4.22)	0.59 (0.34 to 1.04)	0.066
		CT	42	286 (100 to UD)	46	247 (89 to UD)	1.13 (0.62 to 2.05)		
		CC	11	UD (159 to UD)	20	UD (60 to UD)	1.74 (0.46 to 6.59)		
	rs11568820	CC	48	UD (100 to UD)	87	231 (80 to UD)	1.75 (1.04 to 2.95)	1.03 (0.62 to 1.69)	0.92
		CT	30	UD (129 to UD)	29	328 (130 to UD)	1.14 (0.53 to 2.45)		
		TT	23	339 (125 to UD)	17	172 (58 to UD)	1.80 (0.67 to 4.80)		
	rs7976091	CC	48	UD (100 to UD)	87	234 (85 to UD)	1.71 (1.01 to 2.89)	0.99 (0.60 to 1.64)	0.98
		CT	29	UD (107 to UD)	28	328 (130 to UD)	1.02 (0.47 to 2.20)		
		TT	22	339 (125 to UD)	17	172 (58 to UD)	1.46 (0.52 to 4.14)		
	rs2238136	CC	60	UD (129 to UD)	86	262 (75 to UD)	1.54 (0.93 to 2.55)	1.15 (0.59 to 2.24)	0.69
		CT	38	UD (107 to UD)	40	329 (86 to UD)	1.53 (0.78 to 3.02)		
		TT	3*	-	10	88 (30 to UD)	-		
	rs1544410	CC	34	UD (100 to UD)	50	203 (87 to UD)	2.21 (1.09 to 4.49)	0.63 (0.36 to 1.13)	0.12
		CT	46	UD (171 to UD)	64	234 (55 to UD)	1.84 (1.06 to 3.20)		
		TT	18	UD (118 to UD)	15	UD (UD to UD)	0.51 (0.13 to 1.98)		
	rs2228570	GG	45	UD (107 to UD)	61	262 (75 to UD)	1.51 (0.83 to 2.73)	1.13 (0.65 to 1.97)	0.67
		AG	42	297 (176 to UD)	57	234 (87 to UD)	1.30 (0.73 to 2.29)		
		AA	13	UD (100 to UD)	14	89 (47 to UD)	3.51 (0.95 to 13.00)		
	rs2853559	GG	43	UD (154 to UD)	55	210 (50 to UD)	1.84 (1.02 to 3.32)	0.71 (0.40 to 1.26)	0.24
		AG	48	UD (83 to UD)	63	276 (88 to UD)	1.36 (0.76 to 2.42)		
		AA	10	280 (171 to UD)	18	301 (60 to UD)	1.33 (0.34 to 5.18)		
	rs7975232	AA	37	284 (118 to UD)	34	UD (75 to UD	0.85 (0.40 to 1.79)	1.80 (1.01 to 3.20)	0.046
		AC	49	UD (125 to UD)	72	194 (59 to UD)	1.84 (1.08 to 3.14)		
		CC	14	UD (217 to UD)	26	164 (87 to UD)	3.21 (1.03 to 10.02)		
	rs7970314	AA	43	UD (100 to UD)	83	247 (88 to UD)	1.51 (0.88 to 2.60)	1.17 (0.73 to 1.88)	0.52
		AG	31	UD (129 to UD)	32	205 (24 to UD)	1.45 (0.71 to 2.97)		
		GG	28	UD (125 to UD)	20	172 (50 to UD)	2.36 (0.97 to 5.79)		
	rs731236	AA	39	UD (154 to UD)	58	220 (87 to UD)	2.10 (1.09 to 4.04)	0.63 (0.36 to 1.11)	0.11
		AG	47	297 (129 to UD)	59	194 (59 to UD)	1.67 (0.98 to 2.83)		
		GG	14	UD (107 to UD)	17	UD (75 to UD)	0.63 (0.17 to 2.30)		

[1] *Adjusted for study group (resident vs. carer); minimisation variables (level of care, size of scheme and season of randomisation); a significant predictor of upper respiratory infection i.e. baseline vitamin D status (<50 nmol/L vs. ≥50 nmol/L); and allocation to study intervention or placebo arm. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for interaction remain significant. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category.*

Abbreviations: SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, CI: Confidence interval, UD: Undefined, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor, CUBN: Cubilin.

Table 8.4. Interaction analysis: Time to first LRI.

Gene	SNP	Geno-type	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within subgroup (95% CI)	Hazard ratio for allocation*geno-type interaction (95% CI)	P value for interaction [1]
CUBN	rs3740165	TT	85	UD (138 to UD)	122	UD (117 to UD)	1.20 (0.78 to 1.85)	0.67 (0.16 to 2.76)	0.58
		TC	12	337 (86 to UD)	8	UD (53 to UD)	0.50 (0.12 to 2.10)		
		CC	1*	-	0	-	-		
CYP24A1	rs2762939	GG	45	UD (137 to UD)	59	UD (105 to UD)	1.05 (0.56 to 1.96)	1.19 (0.67 to 2.11)	0.55
		CG	38	362 (132 to UD)	57	361 (80 to UD)	1.28 (0.66 to 2.46)		
		CC	19	UD (183 to UD)	13	306 (115 to UD)	1.60 (0.43 to 5.95)		
	rs2248137	CC	29	362 (118 to UD)	46	UD (203 to UD)	0.77 (0.37 to 1.60)	1.38 (0.81 to 2.35)	0.23
		CG	44	UD (138 to UD)	60	273 (60 to UD)	1.48 (0.81 to 2.70)		
		GG	28	UD (137 to UD)	26	313 (89 to UD)	1.56 (0.65 to 3.75)		
	rs2762934	GG	67	UD (148 to UD)	89	UD (126 to UD)	0.97 (0.58 to 1.61)	1.36 (0.65 to 2.84)	0.41
		AG	26	346 (114 to UD)	35	273 (58 to UD)	1.46 (0.70 to 3.04)		
		AA	5*	-	5*	-	-		
	rs6013897	TT	58	362 (109 to UD)	83	UD (127 to UD)	0.84 (0.50 to 1.43)	2.79 (1.38 to 5.63)	0.004
		AT	39	UD (213 to UD)	40	276 (115 to UD)	1.93 (0.91 to 4.09)		
		AA	4*	-	8	58 (24 to 273)	-		
CYP27A1	rs17470271	AA	42	UD (167 to UD)	50	257 (56 to UD)	1.44 (0.76 to 2.73)	0.79 (0.46 to 1.36)	0.40
		AT	43	362 (85 to UD)	59	UD (105 to UD)	0.80 (0.44 to 1.45)		
		TT	14	UD (227 to UD)	24	UD (170 to UD)	0.83 (0.30 to 2.31)		
CYP27B1	rs4646537	TT	88	UD (125 to UD)	114	363 (109 to UD)	1.18 (0.77 to 1.79)	1.33 (0.23 to 7.71)	0.75
		GT	9	UD (UD to UD)	15	UD (306 to UD)	2.53 (0.15 to 41.03)		
		GG	0	-	0	-	-		
	rs4646536	AA	44	UD (125 to UD)	71	306 (88 to UD)	1.52 (0.82 to 2.82)	0.78 (0.43 to 1.41)	0.41
		AG	46	362 (148 to UD)	44	UD (141 to UD)	1.06 (0.56 to 2.00)		
		GG	11	UD (132 to UD)	19	UD (203 to UD)	0.91 (0.19 to 4.32)		
CYP2R1	rs10500804	TT	49	UD (183 to UD)	56	363 (89 to UD)	1.72 (0.87 to 3.38)	0.70 (0.40 to 1.22)	0.21
		GT	40	294 (85 to UD)	59	UD (115 to UD)	0.89 (0.48 to 1.63)		
		GG	12	346 (138 to UD)	20	UD (88 to UD)	0.97 (0.33 to 2.88)		
	rs2060793	GG	34	346 (138 to UD)	43	361 (88 to UD)	1.05 (0.52 to 2.11)	0.99 (0.56 to 1.74)	0.98
		AG	49	UD (137 to UD)	64	UD (109 to UD)	1.09 (0.60 to 1.97)		
		AA	19	UD (132 to UD)	26	363 (89 to UD)	1.72 (0.57 to 5.19)		
	rs10766197	GG	46	UD (154 to UD)	48	262 (80 to UD)	3.18 (1.50 to 6.74)	0.51 (0.30 to 0.92)	0.025
		AG	39	284 (85 to UD)	61	UD (109 to UD)	0.78 (0.43 to 1.43)		
		AA	16	346 (111 to UD)	16	UD (119 to UD)	0.91 (0.32 to 2.55)		
CYP3A4	rs2740574	AA	72	UD (132 to UD)	109	UD (88 to UD)	1.24 (0.78 to 1.96)	0.99 (0.53 to 1.83)	0.97
		AG	13	UD (137 to UD)	16	276 (109 to UD)	1.51 (0.46 to 4.94)		
		GG	16	UD (154 to UD)	8	306 (42 to UD)	1.35 (0.33 to 5.45)		
DBP	rs7041	AA	31	UD (234 to UD)	36	224 (52 to UD)	2.85 (1.21 to 6.71)	2.03 (1.16 to 3.56)	0.014
		AC	44	UD (118 to UD)	61	UD (165 to UD)	0.97 (0.51 to 1.84)		
		CC	24*	-	36	UD (115 to UD)	-		
	rs4588	GG	61	362 (137 to UD)	76	UD (89 to UD)	1.02 (0.61 to 1.71)	1.35 (0.69 to 2.66)	0.38
		GT	35	UD (132 to UD)	51	363 (117 to UD)	1.66 (0.82 to 3.35)		
		TT	5	UD (162 to UD)	8	UD (109 to UD)	0.48 (0.04 to 6.42)		
	rs12512631	TT	45	362 (132 to UD)	67	313 (88 to UD)	1.38 (0.77 to 2.45)	0.83 (0.45 to 1.55)	0.57
		CT	46	UD (125 to UD)	54	363 (105 to UD)	1.60 (0.82 to 3.12)		
		CC	9	UD (138 to UD)	14	UD (271 to UD)	0.09 (0.01 to 1.22)		
	rs2070741	TT	80	UD (125 to UD)	108	UD (117 to UD)	1.00 (0.64 to 1.58)	0.50 (0.17 to 1.49)	0.22
		TG	17	UD (346 to UD)	19	306 (59 to UD)	2.23 (0.70 to 7.05)		
		GG	1	-	2	-	-		
	rs2298849	AA	51	UD (109 to UD)	81	363 (115 to UD)	1.12 (0.65 to 1.94)	1.06 (0.60 to 1.86)	0.84
		AG	39	UD (154 to UD)	36	UD (88 to UD)	1.15 (0.54 to 2.43)		
		GG	11	339 (114 to UD)	14	151 (40 to UD)	1.41 (0.45 to 4.43)		
	rs16846876	AA	49	339 (118 to UD)	67	UD (165 to UD)	0.61 (0.34 to 1.08)	2.57 (1.32 to 5.02)	0.006
		AT	46	UD (167 to UD)	57	234 (85 to UD)	2.42 (1.26 to 4.66)		
		TT	7	UD (162 to UD)	11	188 (80 to UD)	0.84 (0.15 to 4.78)		

Table 8.4 continued.

Gene	SNP	Geno-type	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within subgroup (95% CI)	Hazard ratio for allocation*geno-type interaction (95% CI)	P value for interaction [1]
DHCR7	rs3829251	GG	68	UD (138 to UD)	97	361 (85 to UD)	1.45 (0.88 to 2.38)	0.54 (0.25 to 1.16)	0.11
		AG	27	UD (137 to UD)	28	UD (234 to UD)	0.82 (0.33 to 2.03)		
		AA	3	-	5	232 (117 to UD)	-		
	rs12785878	TT	41	UD (167 to UD)	74	UD (136 to UD)	1.00 (0.54 to 1.85)	1.01 (0.58 to 1.73)	0.98
		GT	40	UD (162 to UD)	42	273 (56 to UD)	1.67 (0.87 to 3.20)		
		GG	20	234 (39 to UD)	18	232 (50 to UD)	0.69 (0.26 to 1.82)		
LRP2	rs3755166	GG	46	337 (132 to UD)	51	363 (136 to UD)	0.97 (0.54 to 1.74)	1.21 (0.66 to 2.23)	0.53
		AG	42	UD (167 to UD)	70	361 (85 to UD)	1.39 (0.75 to 2.57)		
		AA	14	UD (284 to UD)	14	UD (273 to UD)	0.36 (0.08 to 1.69)		
RXRA	rs7861779	GG	55	UD (148 to UD)	84	UD (109 to UD)	1.20 (0.69 to 2.08)	0.92 (0.52 to 1.62)	0.76
		GA	29	284 (118 to UD)	32	363 (136 to UD)	0.72 (0.35 to 1.45)		
		AA	11	UD (234 to UD)	13	363 (105 to UD)	3.26 (0.66 to 16.22)		
	rs9409929	GG	59	UD (125 to UD)	64	UD (127 to UD)	0.96 (0.54 to 1.71)	1.15 (0.65 to 2.05)	0.63
		AG	32	UD (280 to UD)	56	262 (53 to UD)	2.34 (1.13 to 4.88)		
		AA	10	167 (65 to UD)	13	UD (210 to UD)	0.66 (0.10 to 4.54)		
VDR	rs4334089	GG	41	UD (162 to UD)	75	363 (89 to UD)	1.66 (0.91 to 3.01)	0.82 (0.47 to 1.42)	0.47
		AG	40	337 (132 to UD)	39	UD (170 to UD)	0.63 (0.30 to 1.34)		
		AA	20	346 (125 to UD)	17	273 (59 to UD)	1.31 (0.43 to 3.96)		
	rs10783219	AA	55	UD (118 to UD)	54	306 (59 to UD)	1.23 (0.67 to 2.26)	1.08 (0.58 to 2.01)	0.81
		AT	36	UD (167 to UD)	51	363 (126 to UD)	1.38 (0.71 to 2.67)		
		TT	9	UD (302 to UD)	25	UD (109 to UD)	0.80 (0.20 to 3.16)		
	rs4516035	TT	45	UD (154 to UD)	62	313 (85 to UD)	1.43 (0.78 to 2.63)	0.64 (0.36 to 1.14)	0.13
		CT	42	UD (132 to UD)	46	363 (127 to UD)	1.05 (0.54 to 2.03)		
		CC	11	162 (118 to UD)	20	UD (262 to UD)	0.68 (0.20 to 2.31)		
	rs11568820	CC	48	UD (148 to UD)	87	UD (117 to UD)	1.31 (0.76 to 2.27)	0.99 (0.59 to 1.66)	0.97
		CT	30	UD (109 to UD)	29	276 (165 to UD)	1.01 (0.45 to 2.25)		
		TT	23	UD (125 to UD)	17	306 (60 to UD)	1.69 (0.57 to 5.05)		
	rs7976091	CC	48	UD (148 to UD)	87	UD (119 to UD)	1.28 (0.74 to 2.22)	1.04 (0.62 to 1.76)	0.88
		CT	29	346 (86 to UD)	28	UD (165 to UD)	0.88 (0.40 to 1.98)		
		TT	22	UD (154 to UD)	17	273 (58 to UD)	1.66 (0.59 to 4.70)		
	rs2238136	CC	60	UD (137 to UD)	86	313 (105 to UD)	1.30 (0.77 to 2.20)	0.84 (0.41 to 1.71)	0.63
		CT	38	UD (138 to UD)	40	UD (117 to UD)	0.96 (0.48 to 1.91)		
		TT	3*	-	10	UD (89 to UD)	-		
	rs1544410	CC	34	UD (162 to UD)	50	UD (126 to UD)	1.40 (0.66 to 2.95)	0.79 (0.44 to 1.40)	0.41
		CT	46	337 (111 to UD)	64	313 (85 to UD)	1.11 (0.63 to 1.93)		
		TT	18	UD (137 to UD)	15	UD (273 to UD)	0.83 (0.22 to 3.04)		
	rs2228570	GG	45	UD (111 to UD)	61	313 (88 to UD)	1.50 (0.81 to 2.79)	0.71 (0.39 to 1.31)	0.27
		AG	42	UD (213 to UD)	57	UD (141 to UD)	1.03 (0.54 to 1.98)		
		AA	13	280 (118 to UD)	14	UD (58 to UD)	1.39 (0.34 to 5.61)		
	rs2853559	GG	43	346 (154 to UD)	55	271 (85 to UD)	1.44 (0.80 to 2.59)	0.68 (0.36 to 1.28)	0.23
		AG	48	UD (137 to UD)	63	UD (126 to UD)	1.09 (0.57 to 2.07)		
		AA	10	280 (118 to UD)	18	UD (141 to UD)	0.52 (0.14 to 1.99)		
	rs7975232	AA	37	UD (132 to UD)	34	UD (273 to UD)	0.64 (0.29 to 1.45)	1.73 (0.97 to 3.10)	0.065
		AC	49	362 (114 to UD)	72	306 (88 to UD)	1.26 (0.72 to 2.19)		
		CC	14	UD (UD to UD)	26	232 (89 to UD)	3.87 (1.08 to 13.88)		
	rs7970314	AA	43	UD (148 to UD)	83	UD (126 to UD)	1.15 (0.64 to 2.07)	1.19 (0.72 to 1.94)	0.50
		AG	31	294 (111 to UD)	32	363 (59 to UD)	0.90 (0.43 to 1.88)		
		GG	28	UD (125 to UD)	20	257 (50 to UD)	1.33 (0.55 to 3.23)		
	rs731236	AA	39	UD (154 to UD)	58	UD (119 to UD)	1.58 (0.77 to 3.22)	0.68 (0.38 to 1.23)	0.21
		AG	47	302 (111 to UD)	59	306 (85 to UD)	1.18 (0.67 to 2.09)		
		GG	14	UD (137 to UD)	17	UD (273 to UD)	0.82 (0.21 to 3.21)		

[1] *Adjusted for stratification factors i.e. Percent predicted forced expiratory volume in 1 second (<50% vs. ≥50%) and inclusion in vs. exclusion from sputum induction sub-study; significant predictors of upper respiratory infection i.e. Age (<70 years vs. ≥70 years), and smoking status (current vs. non-current); and allocation to study intervention or placebo arm. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for interaction remain significant.*
** Genotype could not be analysed due to <5 participants and/or too few events within this sub category.*

Abbreviations: SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, CI: Confidence interval, UD: Undefined, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor, CUBN: Cubilin.

8.3. Discussion.

In this chapter I have investigated a link between known variants in common vitamin D pathway genes and risk of URI and LRI in older adults, both independently and via modification of vitamin D supplementation effect. My panel of 35 SNP did not predict risk of these outcomes after correction for multiple comparisons testing.

Previous studies have yet to investigate whether variation in vitamin D related genes affect risk of ARI in older adults, though two groups have reported a main effect association between *FokI* mutation and risk of severe RSV infection and LRI, in children (202, 340). In both cases the mutant allele (f) associated with increased risk. The number of studies which have reported an association between vitamin D related SNP and various infectious disease outcomes make a strong case for the importance of genetic variation in the vitamin D pathway on risk of ARI. These include associations with: Tuberculosis risk, or resolution of inflammation during adjunctive vitamin D therapy (*FokI* [rs10735810/rs2228570], *BsmI* [rs1544410] and *TaqI* [rs731236] in *VDR*, and rs7041/rs4588 in *DBP*) (184, 203, 205, 346, 347); risk of hepatitis C (*ApaI* [rs7975232] in *VDR*, rs10877012 in *CYP27B1*) (133, 348); risk of HIV infection (rs4516035 in *VDR*) (349); risk of rubella (*Cdx2* [rs11568820] and rs7970314 in *VDR*) (350); and risk of Periodontitis (*BsmI* [rs1544410], ApaI [rs7975232] and TaqI [rs731236] in *VDR*) (351) (352). However, none of these studies have focused specifically on older adult populations. Further investigation of the effects of vitamin D supplementation in prevention of ARI in older adults is clearly needed, and is something we will address in our current IPD meta-analysis, but there are a range of proposed factors surrounding age-related immune system decline which may be responsible for the lack of effect I have seen in this study. These include age-related impairment of neutrophil chemotaxis and antigen presentation by macrophages (91, 92) (93); the progressive increase in production of pro-inflammatory cytokines ("inflammaging") (94); attenuation of adaptive immune responses via reduced dendritic cell-mediated stimulation of T and B cells (95); impairment of T cell activation via dysregulation of signal transduction proteins (96, 97); reduced

naïve lymphocytes populations due to thymic involution (98); decreased progenitor B cell production in bone marrow (99); and accumulation of anergic effector memory CD8+ T cells (100). All of these factors could potentially affect the mechanisms by which vitamin D modulates immune responses to respiratory pathogens, and dampen its ability to offer protection against ARI in older adults, which may explain null reports from previous clinical trials in this study population (273) (267) (344) and the non-significant effect of genetic variants I report in this study.

8.3.1. Study strengths.

To my knowledge, this is the first study to comprehensively investigate genetic variation in the vitamin D pathway on risk of URI and LRI in the older adult population. The majority of participants were vitamin D deficient at baseline and using intermittent bolus dosing a high level of compliance was achieved, which translated to a good average level of repletion in the active arm. Participants were well characterised for possible environmental determinants of ARI and vitamin D status, which allowed wide control for factors which might confound the association between genetic variation and my investigated outcomes. Another strength of this study was the comprehensive panel of SNP which capture the current body of positive genetic association findings between vitamin D pathway variants and vitamin D metabolite / non-skeletal disease outcomes. Finally, 25(OH)D concentrations were measured with the gold standard assay (LC-MS/MS) in a laboratory that participated in the international vitamin D external quality assurance scheme (www.deqas.org/).

8.3.2. Study limitations.

One limitation of this study arises from the use of data from a clinical trial which was powered to detect respiratory outcomes in the study population as a whole, but was not powered to detect the same outcomes across ranging frequencies of SNP genotypes. This may have resulted in type II error, especially when correcting for multiple comparison testing. One further feature of the clinical trial

which may have limited the potential to find associations in this study was the use of an intermittent monthly bolus dosing regimen of vitamin D supplementation in addition to a small daily dose, which may have caused a sharp enough spike in vitamin D levels to upregulate synthesis of CYP24A1 enzyme, which is responsible for breaking down both 25(OH)D and $1,25(OH)_2D$ (343). This may have resulted in lower extra-renal concentrations of the active metabolite in the active arm than the control arm, as the control arm received only the small daily dose of vitamin D.

8.4. Conclusions.

In this study, none of the 35 SNP in 11 vitamin D related genes showed a significant main effects association with ARI in older adults, nor did they modify the effects of vitamin D supplementation for the prevention of ARI, which suggests vitamin D status does not impact risk of ARI in this population. Whilst the analysis was well controlled for potential cofounding factors, a lack of statistical power, or type II error from strict multiple comparisons testing may also be responsible for these null results, as may the use of an intermittent bolus dosing regimen of vitamin D which could have resulted in lower than optimal concentrations of localised $1,25(OH)_2D$ in pulmonary tissue. Future research should be conducted to investigate the impact of genetic variants in this population group, in large clinical trials which deliver a suitably large daily dosing regimen.

9. Genetic variants in the vitamin D pathway and susceptibility to acute respiratory infections in patients with COPD.

In this chapter I present findings of an investigation into the relationship between single nucleotide polymorphisms in the vitamin D pathway and the risk of upper respiratory infection and acute exacerbation in patients with COPD who participated in the ViDiCO trial (described in Methods, section 2.2.1). This comprises an investigation of main effects association between SNP and risk of URI / exacerbation, and an investigation of effect modification of SNP on vitamin D supplementation in prevention of these outcomes.

9.1. Introduction.

There is a high prevalence of vitamin D deficiency in chronic obstructive pulmonary disease (COPD) patients who are at higher than normal risk for upper respiratory infections (URI) (323). URI commonly lead to life-threatening acute symptom exacerbations in COPD patients and there are currently no interventions to treat them (353). Two clinical trials of vitamin D supplementation in the prevention of URI and COPD exacerbation have been conducted to date and both found no protective effect of supplementation in the study group as a whole, but did report reduced risk of moderate to severe disease exacerbation in subgroups who were profoundly vitamin D deficient at baseline (210, 272). Another potential subgroup effect which has not been comprehensively explored is that of genotype as a determinant of outcome risk, either directly or as modifier of the effects of vitamin D supplementation. Polymorphic genes have been identified within pathways of vitamin D metabolism, transport, and signalling which associate with variation in vitamin D status, and with a striking array of disease outcomes. Variation in vitamin D binding protein (DBP) is the most widely reported determinant of vitamin D status, whilst variation in vitamin D receptor protein (VDR) has been highlighted as the major determinant of disease outcomes (335). A limited number of findings link single nucleotide polymorphisms (SNP) in *DBP* to COPD: a large cohort study

conducted in Belgium found rs7041 to associate with risk of vitamin D deficiency and risk of COPD (323), whilst a Norwegian case-control study reported an association (which they replicated in a second study population) between rs1155563 / rs17467825 and clinical markers of COPD severity, namely lung function tests: percent predicted forced expiratory volume in 1 second (% FEV_1) and FEV_1 to forced vital capacity (FVC) ratio (336). Research investigating variation in *VDR* in association with COPD is lacking, however several findings relating to respiratory infection and disease have been made: *FokI* mutation (rs10735810/rs2228570) has been found to associate with risk of respiratory syncytial virus (RSV) infection and acute lower respiratory infection (LRI) in child cohorts from the Netherlands (339), South Africa (340), and Canada (202); and with risk of tuberculosis in Gujarati Asians residing in London (346), Chinese Hans (203), and North Central Indian Castes (347). Furthermore, *BsmI* mutation (rs1544410) has been found to associate with risk of smear-positive, multi-drug-resistant tuberculosis in three Central Indian populations (347). Besides these main effect associations, findings of genetic effect modification have also been reported: *TaqI* mutation (rs731236) in *VDR* has been shown to modify the effects of vitamin D supplementation on time to sputum culture conversion in patients receiving treatment for tuberculosis, in the UK (205); risk of advanced colorectal adenoma, in a UK clinical trial (341); and *FokI* mutation has been reported to modify the effect of vitamin D supplementation on treatment of Parkinson's disease, in a clinical trial conducted in Japan (342).

The possible mechanisms by which polymorphic vitamin D pathway genes may influence risk of ARI and exacerbations of COPD are numerous, and not restricted to *DBP* and *VDR*. The investigation of genetic determinants of health outcomes in COPD patients is lacking. Therefore in this chapter I will present results of an analysis of 35 SNP in 11 vitamin D pathway genes which have previously been linked to vitamin D status and/or risk of non-skeletal disease, for a main effects association with URI and COPD exacerbation, and as potential effect-modifiers of vitamin D supplementation on risk of these outcomes.

9.2. Results.

9.2.1. Main effects: does vitamin D pathway genotype influence risk of URI or COPD exacerbation, independent of vitamin D supplementation?

Results of main effects analyses are presented in Table 9.1 and Table 9.2. Multivariable Cox regression analysis (described in Methods, section 2.4.2) identified the following SNP genotypes to associate with risk of URI: referent to GG genotype for rs4334089 (*VDR*), AG genotype associated with a 41% higher risk, and AA genotype associated with a 77% higher risk (P value for trend = 0.015); referent to CC genotype for rs11568820 (*VDR*), CT genotype associated with a 41% higher risk, and TT genotype associated with a 112% higher risk (P value for trend = 0.013); referent to CC genotype for rs7976091 (*VDR*), CT genotype associated with a 38% higher risk, and TT genotype associated with a 124% higher risk (P value for trend = 0.011); finally, referent to AA genotype for rs7970314 (*VDR*), AG genotype associated with a 34% higher risk, and GG genotype associated with a 92% higher risk (P value for trend = 0.024).

Multivariable Cox regression analysis also identified one SNP to show an association with risk of COPD exacerbation: referent to CC genotype for rs2238136 (*VDR*), CT genotype associated with a 26% lower risk, and TT genotype associated with a 60% lower risk (P value for trend = 0.026) (Table 9.2). However, after correction for multiple comparisons testing (Bejamini & Hochberg method with a false discovery rate of 5%) none of these main effects associations remained significant.

Table 9.1. Main effects: Time to first URI.

Gene	SNP	Genotype	N	Days at risk (IQR)	Univariable Hazard Ratio (95% CI)	P value	Multivariable Hazard Ratio (95% CI)	P value [1]
CUBN	rs3740165	TT	223	176 (28 to UD)	referent	0.26	referent	0.38
		TC	12	156 (54 to UD)	0.79 (0.37 to 1.69)		0.87 (0.40 to 1.88)	
		CC	1*	-	-		-	
CYP24A1	rs2762939	GG	133	217 (26 to UD)	referent	0.15	referent	0.20
		CG	93	137 (33 to 372)	1.24 (0.89 to 1.74)		1.29 (0.92 to 1.82)	
		CC	11	120 (61 to 344)	1.40 (0.70 to 2.80)		1.19 (0.59 to 2.42)	
	rs2248137	CC	86	217 (27 to 374)	referent	0.41	referent	0.79
		CG	121	136 (35 to UD)	1.15 (0.82 to 1.63)		1.24 (0.88 to 1.76)	
		GG	30	UD (33 to UD)	0.65 (0.36 to 1.17)		0.74 (0.41 to 1.33)	
	rs2762934	GG	167	203 (27 to UD)	referent	0.46	referent	0.25
		AG	65	144 (33 to 374)	1.20 (0.84 to 1.70)		1.22 (0.85 to 1.75)	
		AA	6	62 (42 to UD)	0.96 (0.35 to 2.62)		1.30 (0.47 to 3.62)	
	rs6013897	TT	156	178 (27 to UD)	referent	0.91	referent	0.83
		AT	67	202 (32 to UD)	0.88 (0.61 to 1.29)		0.89 (0.61 to 1.32)	
		AA	12	92 (54 to 239)	1.31 (0.68 to 2.51)		1.34 (0.69 to 2.59)	
CYP27A1	rs17470271	AA	90	200 (50 to 374)	referent	0.82	referent	0.64
		AT	108	171 (26 to UD)	0.99 (0.70 to 1.41)		1.14 (0.79 to 1.63)	
		TT	40	152 (27 to UD)	1.07 (0.67 to 1.70)		1.08 (0.68 to 1.72)	
CYP27B1	rs4646537	TT	220	176 (32 to UD)	referent	0.71	referent	0.87
		GT	17	88 (26 to UD)	1.12 (0.61 to 2.07)		1.05 (0.57 to 1.96)	
		GG	0*	-	-		-	
	rs4646536	AA	95	159 (26 to UD)	referent	0.55	referent	0.35
		AG	113	176 (35 to UD)	1.05 (0.74 to 1.49)		1.12 (0.79 to 1.59)	
		GG	25	147 (18 to UD)	1.20 (0.70 to 2.06)		1.28 (0.74 to 2.20)	
CYP2R1	rs10500804	TT	80	137 (28 to UD)	referent	0.21	referent	0.57
		GT	115	202 (32 to UD)	0.88 (0.62 to 1.26)		0.99 (0.69 to 1.43)	
		GG	42	261 (43 to UD)	0.74 (0.46 to 1.20)		0.85 (0.51 to 1.40)	
	rs2060793	GG	80	197 (28 to UD)	referent	0.35	referent	0.91
		AG	115	203 (32 to UD)	1.11 (0.77 to 1.59)		0.96 (0.66 to 1.40)	
		AA	42	117 (26 to UD)	1.25 (0.78 to 1.99)		1.04 (0.65 to 1.68)	
	rs10766197	GG	67	198 (45 to UD)	referent	0.86	referent	0.66
		AG	116	159 (27 to UD)	1.18 (0.81 to 1.72)		1.34 (0.91 to 1.98)	
		AA	51	261 (35 to UD)	0.93 (0.58 to 1.49)		1.05 (0.64 to 1.73)	
CYP3A4	rs2740574	AA	214	171 (31 to UD)	referent	0.49	referent	0.30
		AG	23	212 (58 to UD)	0.82 (0.46 to 1.45)		0.73 (0.41 to 1.31)	
		GG	0*	-	-		-	
DBP	rs7041	CC	57	203 (44 to UD)	referent	0.89	referent	0.45
		AC	125	178 (26 to UD)	1.14 (0.77 to 1.70)		1.21 (0.81 to 1.81)	
		AA	52	144 (53 to UD)	1.03 (0.64 to 1.65)		1.19 (0.73 to 1.93)	
	rs4588	GG	112	216 (44 to UD)	referent	0.17	referent	0.13
		GT	107	171 (22 to UD)	1.18 (0.84 to 1.65)		1.26 (0.9 to 1.78)	
		TT	20	119 (53 to 176)	1.42 (0.81 to 2.49)		1.39 (0.79 to 2.46)	
	rs12512631	TT	98	144 (47 to 374)	referent	0.58	referent	0.22
		CT	114	212 (25 to UD)	0.89 (0.63 to 1.25)		0.81 (0.58 to 1.15)	
		CC	23	203 (64 to UD)	0.92 (0.53 to 1.59)		0.76 (0.44 to 1.33)	
	rs2070741	TT	205	173 (28 to UD)	referent	0.46	referent	0.091
		TG	27	197 (47 to UD)	0.86 (0.51 to 1.48)		1.09 (0.63 to 1.89)	
		GG	3*	-	-		-	
	rs2298849	AA	159	203 (33 to UD)	referent	0.14	referent	0.067
		AG	73	178 (27 to UD)	1.08 (0.76 to 1.53)		1.13 (0.79 to 1.62)	
		GG	7	64 (27 to 144)	2.56 (1.18 to 5.54)		3.01 (1.37 to 6.60)	
	rs16846876	AA	108	202 (28 to UD)	referent	0.58	Referent	0.41
		AT	109	197 (35 to UD)	1.02 (0.72 to 1.43)		1.13 (0.80 to 1.59)	
		TT	20	144 (32 to 374)	1.23 (0.70 to 2.15)		1.21 (0.68 to 2.13)	
DHCR7	rs3829251	GG	181	203 (42 to UD)	referent	0.12	referent	0.073

Table 9.1 continued.

Gene	SNP	Genotype	N	Days at risk (IQR)	Univariable Hazard Ratio (95% CI)	P value	Multivariable Hazard Ratio (95% CI)	P value [1]
		AG	49	117 (26 to UD)	1.30 (0.88 to 1.90)		1.33 (0.91 to 1.96)	
		AA	6	27 (22 to 123)	1.65 (0.61 to 4.49)		1.88 (0.68 to 5.24)	
	rs12785878	TT	148	198 (35 to UD)	referent	0.46	referent	0.80
		GT	75	171 (28 to UD)	1.09 (0.77 to 1.54)		1.00 (0.70 to 1.42)	
		GG	14	54 (25 to UD)	1.26 (0.64 to 2.50)		1.15 (0.58 to 2.31)	
LRP2	rs3755166	GG	82	178 (28 to UD)	referent	0.84	referent	0.93
		AG	108	171 (33 to 374)	1.07 (0.75 to 1.54)		1.04 (0.72 to 1.50)	
		AA	48	198 (32 to UD)	0.93 (0.59 to 1.47)		0.97 (0.61 to 1.54)	
RXRA	rs7861779	GG	171	173 (31 to UD)	referent	0.98	referent	0.61
		GA	54	197 (26 to UD)	0.95 (0.65 to 1.41)		0.85 (0.58 to 1.27)	
		AA	5	136 (92 to 159)	1.23 (0.45 to 3.33)		1.13 (0.41 to 3.14)	
	rs9409929	GG	98	197 (58 to UD)	referent	0.054	referent	0.10
		AG	120	171 (26 to UD)	1.25 (0.89 to 1.76)		1.32 (0.93 to 1.87)	
		AA	18	77 (21 to 300)	1.74 (0.97 to 3.14)		1.43 (0.78 to 2.60)	
VDR	rs4334089	GG	121	239 (62 to UD)	referent	0.013	referent	0.015
		AG	97	123 (21 to UD)	1.46 (1.04 to 2.04)		1.41 (0.99 to 2.00)	
		AA	20	47 (15 to 374)	1.68 (0.96 to 2.94)		1.77 (1.00 to 3.13)	
	rs10783219	AA	98	137 (22 to UD)	referent	0.22	referent	0.13
		AT	111	217 (43 to UD)	0.83 (0.59 to 1.17)		0.80 (0.57 to 1.13)	
		TT	24	212 (50 to UD)	0.75 (0.41 to 1.35)		0.70 (0.38 to 1.28)	
	rs4516035	TT	79	200 (33 to UD)	referent	0.97	referent	0.80
		CT	127	143 (25 to 374)	1.19 (0.84 to 1.70)		1.13 (0.79 to 1.61)	
		CC	30	226 (57 to UD)	0.85 (0.47 to 1.51)		0.82 (0.46 to 1.46)	
	rs11568820	CC	138	216 (53 to UD)	referent	0.050	referent	0.013
		CT	877	137 (22 to UD)	1.26 (0.90 to 1.77)		1.41 (0.99 to 2.00)	
		TT	11	27 (15 to UD)	1.92 (0.93 to 3.98)		2.12 (1.01 to 4.45)	
	rs7976091	CC	139	217 (53 to UD)	referent	0.042	referent	0.011
		CT	88	143 (22 to UD)	1.25 (0.90 to 1.75)		1.38 (0.97 to 1.95)	
		TT	12	27 (15 to 152)	1.99 (1.00 to 3.96)		2.24 (1.11 to 4.51)	
	rs2238136	CC	121	154 (23 to UD)	referent	0.53	referent	0.40
		CT	95	217 (59 to 374)	0.92 (0.66 to 1.29)		0.88 (0.63 to 1.23)	
		TT	16	156 (18 to UD)	0.84 (0.40 to 1.74)		0.82 (0.39 to 1.71)	
	rs1544410	CC	85	217 (35 to UD)	referent	0.17	referent	0.19
		CT	114	154 (41 to 374)	1.18 (0.82 to 1.69)		1.15 (0.80 to 1.66)	
		TT	39	143 (19 to 348)	1.37 (0.86 to 2.19)		1.38 (0.86 to 2.22)	
	rs2228570	GG	94	216 (58 to UD)	referent	0.22	referent	0.24
		AG	108	143 (27 to 374)	1.25 (0.88 to 1.78)		1.24 (0.87 to 1.77)	
		AA	35	137 (20 to UD)	1.28 (0.78 to 2.10)		1.26 (0.77 to 2.08)	
	rs2853559	GG	79	171 (27 to 374)	referent	0.51	referent	0.37
		AG	115	159 (28 to UD)	1.08 (0.75 to 1.54)		1.02 (0.71 to 1.46)	
		AA	39	231 (57 to UD)	0.79 (0.47 to 1.31)		0.76 (0.45 to 1.26)	
	rs7975232	AA	65	143 (32 to UD)	referent	0.26	referent	0.26
		AC	117	176 (28 to UD)	0.90 (0.62 to 1.30)		0.93 (0.64 to 1.36)	
		CC	50	217 (42 to UD)	0.76 (0.48 to 1.22)		0.75 (0.47 to 1.21)	
	rs7970314	AA	130	216 (51 to UD)	referent	0.10	referent	0.024
		AG	93	147 (26 to 374)	1.19 (0.85 to 1.66)		1.34 (0.95 to 1.90)	
		GG	14	27 (15 to UD)	1.72 (0.89 to 3.33)		1.92 (0.98 to 3.78)	
	rs731236	AA	89	217 (42 to UD)	referent	0.35	referent	0.50
		AG	114	154 (31 to 374)	1.20 (0.84 to 1.72)		1.14 (0.80 to 1.64)	
		GG	35	171 (23 to UD)	1.20 (0.74 to 1.96)		1.14 (0.70 to 1.87)	

[1] *Adjusted for stratification factors i.e. Percent predicted forced expiratory volume in 1 second (<50% vs. ≥50%) and inclusion in vs. exclusion from sputum induction sub-study; significant predictors of upper respiratory infection i.e. Age (<70 years vs. ≥70 years), and smoking status (current vs. non-current); and allocation to study intervention or placebo arm. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for interaction remain significant. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category.*

Abbreviations: SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, CI: Confidence interval, UD: Undefined, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor, CUBN: Cubilin.

Table 9.2. Main effects: Time to first exacerbation COPD exacerbation.

Gene	SNP	Genotype	N	Days at risk (IQR)	Univariable Hazard Ratio (95% CI)	P value	Multivariable Hazard Ratio (95% CI)	P value [1]
CUBN	rs3740165	TT	223	UD (151 to UD)	referent	0.60	referent	0.55
		TC	12	UD (98 to UD)	1.01 (0.44 to 2.29)		1.04 (0.45 to 2.38)	
		CC	1*	-	-		-	
CYP24A1	rs2762939	GG	133	345 (68 to UD)	referent	0.55	referent	0.34
		CG	93	329 (103 to UD)	1.01 (0.69 to 1.47)		0.94 (0.64 to 1.38)	
		CC	11	UD (180 to UD)	0.60 (0.22 to 1.64)		0.54 (0.20 to 1.51)	
	rs2248137	CC	86	UD (89 to UD)	referent	0.15	referent	0.22
		CG	121	329 (111 to UD)	1.19 (0.79 to 1.78)		1.12 (0.74 to 1.68)	
		GG	30	153 (30 to UD)	1.53 (0.86 to 2.71)		1.48 (0.83 to 2.64)	
	rs2762934	GG	167	371 (87 to UD)	referent	0.41	referent	0.44
		AG	65	329 (87 to UD)	1.17 (0.78 to 1.74)		1.16 (0.77 to 1.73)	
		AA	6	261 (163 to UD)	1.24 (0.46 to 3.40)		1.23 (0.44 to 3.41)	
	rs6013897	TT	156	342 (90 to UD)	referent	0.93	referent	0.77
		AT	67	369 (67 to UD)	1.00 (0.66 to 1.52)		1.05 (0.69 to 1.62)	
		AA	12	224 (89 to UD)	0.95 (0.41 to 2.18)		1.09 (0.47 to 2.51)	
CYP27A1	rs17470271	AA	90	251 (72 to UD)	referent	0.096	referent	0.21
		AT	108	369 (120 to UD)	0.74 (0.50 to 1.10)		0.78 (0.52 to 1.16)	
		TT	40	UD (87 to UD)	0.67 (0.39 to 1.17)		0.75 (0.43 to 1.31)	
CYP27B1	rs4646537	TT	220	342 (89 to UD)	referent	0.78	referent	0.89
		GT	17	261 (123 to UD)	0.90 (0.44 to 1.85)		0.95 (0.46 to 1.96)	
		GG	0*	-	-		-	
	rs4646536	AA	95	369 (95 to UD)	referent	0.86	referent	0.94
		AG	113	263 (67 to UD)	1.33 (0.90 to 1.97)		1.29 (0.87 to 1.91)	
		GG	25	371 (187 to UD)	0.80 (0.40 to 1.59)		0.74 (0.37 to 1.49)	
CYP2R1	rs10500804	TT	80	329 (69 to UD)	referent	0.81	referent	0.59
		GT	115	350 (77 to UD)	1.03 (0.68 to 1.55)		1.14 (0.68 to 1.91)	
		GG	42	315 (125 to UD)	0.92 (0.53 to 1.57)		1.18 (0.68 to 2.02)	
	rs2060793	GG	80	368 (125 to UD)	referent	0.78	referent	0.69
		AG	115	263 (67 to UD)	1.29 (0.85 to 1.95)		1.09 (0.64 to 1.87)	
		AA	42	UD (85 to UD)	0.99 (0.56 to 1.74)		0.94 (0.53 to 1.66)	
	rs10766197	GG	67	329 (89 to UD)	referent	0.76	referent	0.53
		AG	116	350 (68 to UD)	1.08 (0.70 to 1.66)		1.07 (0.69 to 1.66)	
		AA	51	315 (137 to UD)	0.91 (0.54 to 1.54)		0.83 (0.49 to 1.41)	
CYP3A4	rs2740574	AA	214	351 (89 to UD)	referent	0.37	referent	0.41
		AG	23	274 (44 to UD)	1.30 (0.73 to 2.32)		1.28 (0.71 to 2.30)	
		GG	0*	-	-		-	
DBP	rs7041	CC	57	304 (69 to UD)	referent	0.97	referent	0.96
		AC	125	369 (89 to UD)	0.84 (0.54 to 1.30)		0.91 (0.58 to 1.42)	
		AA	52	252 (103 to UD)	1.00 (0.60 to 1.67)		1.02 (0.61 to 1.71)	
	rs4588	GG	112	329 (85 to UD)	referent	0.85	referent	0.68
		GT	107	369 (73 to UD)	0.97 (0.66 to 1.42)		1.05 (0.72 to 1.55)	
		TT	20	194 (111 to UD)	1.14 (0.60 to 2.18)		1.13 (0.59 to 2.17)	
	rs12512631	TT	98	371 (123 to UD)	referent	0.056	referent	0.14
		CT	114	329 (54 to UD)	1.35 (0.90 to 2.00)		1.32 (0.89 to 1.97)	
		CC	23	180 (77 to UD)	1.66 (0.92 to 3.00)		1.42 (0.78 to 2.58)	
	rs2070741	TT	205	329 (85 to UD)	referent	0.39	referent	0.34
		TG	27	UD (120 to UD)	0.87 (0.48 to 1.58)		0.89 (0.49 to 1.65)	
		GG	3*	-	-		-	
	rs2298849	AA	159	304 (85 to UD)	referent	0.59	referent	0.97
		AG	73	371 (120 to UD)	0.83 (0.55 to 1.24)		0.95 (0.63 to 1.45)	
		GG	7	274 (20 to UD)	1.21 (0.44 to 3.29)		1.24 (0.45 to 3.43)	
	rs16846876	AA	108	304 (62 to UD)	referent	0.20	referent	0.28
		AT	109	351 (111 to UD)	0.85 (0.59 to 1.25)		0.88 (0.61 to 1.29)	
		TT	20	UD (129 to UD)	0.64 (0.30 to 1.34)		0.68 (0.32 to 1.43)	
DHCR7	rs3829251	GG	181	342 (87 to UD)	referent	0.82	referent	0.89

Table 9.2 continued.

Gene	SNP	Genotype	N	Days at risk (IQR)	Univariable Hazard Ratio (95% CI)	P value	Multivariable Hazard Ratio (95% CI)	P value [1]
		AG	49	201 (89 to UD)	1.04 (0.67 to 1.62)		1.12 (0.72 to 1.75)	
		AA	6	UD (233 to UD)	0.64 (0.16 to 2.60)		0.56 (0.14 to 2.29)	
	rs12785878	TT	148	304 (67 to UD)	referent	0.47	referent	0.48
		GT	75	369 (125 to UD)	0.78 (0.52 to 1.17)		0.79 (0.52 to 1.18)	
		GG	14	233 (28 to UD)	1.05 (0.48 to 2.27)		1.05 (0.48 to 2.30)	
LRP2	rs3755166	GG	82	350 (108 to UD)	referent	0.99	referent	0.94
		AG	108	304 (64 to UD)	1.16 (0.77 to 1.75)		1.16 (0.77 to 1.75)	
		AA	48	UD (89 to UD)	0.96 (0.57 to 1.63)		0.98 (0.58 to 1.66)	
RXRA	rs7861779	GG	171	329 (90 to UD)	referent	0.70	referent	0.58
		GA	54	345 (89 to UD)	1.00 (0.64 to 1.55)		1.07 (0.69 to 1.67)	
		AA	5	133 (20 to UD)	1.60 (0.50 to 5.06)		1.41 (0.44 to 4.55)	
	rs9409929	GG	98	371 (123 to UD)	referent	0.58	referent	0.91
		AG	120	252 (56 to UD)	1.40 (0.95 to 2.06)		1.35 (0.91 to 2.00)	
		AA	18	UD (187 to UD)	0.78 (0.35 to 1.74)		0.67 (0.30 to 1.50)	
VDR	rs4334089	GG	121	UD (103 to UD)	referent	0.16	referent	0.24
		AG	97	261 (68 to UD)	1.33 (0.91 to 1.95)		1.28 (0.87 to 1.88)	
		AA	20	329 (57 to 369)	1.33 (0.71 to 2.49)		1.27 (0.68 to 2.40)	
	rs10783219	AA	98	368 (87 to UD)	referent	0.67	referent	0.83
		AT	111	329 (111 to UD)	1.02 (0.69 to 1.49)		0.97 (0.66 to 1.43)	
		TT	24	UD (89 to UD)	0.79 (0.39 to 1.61)		0.94 (0.46 to 1.92)	
	rs4516035	TT	79	371 (67 to UD)	referent	0.80	referent	0.78
		CT	127	301 (90 to UD)	1.13 (0.76 to 1.70)		1.05 (0.70 to 1.57)	
		CC	30	UD (49 to UD)	0.99 (0.52 to 1.90)		1.08 (0.56 to 2.08)	
	rs11568820	CC	138	329 (89 to UD)	referent	0.73	referent	0.83
		CT	877	342 (89 to UD)	1.12 (0.77 to 1.63)		1.13 (0.77 to 1.64)	
		TT	11	UD (57 to UD)	0.94 (0.38 to 2.33)		0.84 (0.33 to 2.12)	
	rs7976091	CC	139	329 (89 to UD)	referent	0.52	referent	0.60
		CT	88	345 (87 to UD)	1.14 (0.78 to 1.65)		1.15 (0.79 to 1.68)	
		TT	12	278 (21 to UD)	1.13 (0.49 to 2.61)		1.01 (0.43 to 2.37)	
	rs2238136	CC	121	278 (57 to UD)	referent	0.052	referent	0.026
		CT	95	368 (111 to UD)	0.81 (0.55 to 1.19)		0.74 (0.50 to 1.08)	
		TT	16	UD (240 to UD)	0.39 (0.14 to 1.07)		0.40 (0.14 to 1.10)	
	rs1544410	CC	85	263 (95 to UD)	referent	0.91	referent	0.85
		CT	114	371 (77 to UD)	0.90 (0.60 to 1.35)		0.91 (0.60 to 1.37)	
		TT	39	263 (95 to UD)	1.08 (0.64 to 1.81)		0.98 (0.58 to 1.67)	
	rs2228570	GG	94	342 (87 to UD)	referent	0.32	referent	0.42
		AG	108	UD (103 to UD)	0.85 (0.57 to 1.27)		0.87 (0.58 to 1.30)	
		AA	35	132 (45 to UD)	1.52 (0.91 to 2.52)		1.39 (0.83 to 2.32)	
	rs2853559	GG	79	369 (72 to UD)	referent	0.21	referent	0.17
		AG	115	350 (111 to UD)	0.98 (0.64 to 1.48)		0.90 (0.59 to 1.37)	
		AA	39	153 (45 to UD)	1.51 (0.90 to 2.54)		1.65 (0.97 to 2.79)	
	rs7975232	AA	65	345 (89 to UD)	referent	0.71	referent	0.97
		AC	117	368 (69 to UD)	0.96 (0.62 to 1.48)		1.04 (0.68 to 1.62)	
		CC	50	369 (123 to UD)	0.90 (0.53 to 1.54)		0.98 (0.57 to 1.68)	
	rs7970314	AA	130	329 (89 to UD)	referent	0.90	referent	0.96
		AG	93	345 (89 to UD)	1.10 (0.76 to 1.61)		1.10 (0.75 to 1.61)	
		GG	14	UD (57 to UD)	0.87 (0.37 to 2.01)		0.83 (0.35 to 1.94)	
	rs731236	AA	89	301 (108 to UD)	referent	0.77	referent	0.93
		AG	114	368 (77 to UD)	0.96 (0.64 to 1.43)		0.93 (0.62 to 1.39)	
		GG	35	329 (73 to UD)	1.13 (0.66 to 1.92)		1.00 (0.58 to 1.73)	

[1] *Adjusted for stratification factors i.e. Percent predicted forced expiratory volume in 1 second (<50% vs. ≥50%) and inclusion in vs. exclusion from sputum induction sub-study; a significant predictor of COPD exacerbation i.e. use of inhaled corticosteroids (yes vs. no); and allocation to study intervention or placebo arm. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for interaction remain significant.* ******* *Genotype could not be analysed due to <5 participants and/or too few events within this sub category.*

Abbreviations: SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, CI: Confidence interval, UD: Undefined, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor A, VDR: Vitamin D receptor, CUBN: Cubilin.

9.2.2. *Interaction analysis: does genotype influence effect of vitamin D supplementation in prevention of URI or COPD exacerbation?*

Results of interaction analyses testing for effect modification are presented in Table 9.3 and Table 9.4. Multivariable Cox regression analysis (described in Methods, section 2.4.3) identified 5 SNP in *VDR* which modify the effect of vitamin D supplementation on risk of URI: Vitamin D supplementation conferred increased risk of URI to individuals with GG genotype for rs4334089 (aHR 1.65; 95% CI 1.00 to 2.70); offered protection from URI to individuals with AG genotype (aHR 0.45; 95% CI 0.27 to 0.76); and had no effect on individuals with AA genotype (aHR 0.90; 95% CI 0.13 to 6.21 – aRHR 0.36; 95% CI 0.22 to 0.60; P value for interaction <0.001). Vitamin D supplementation conferred increased risk of URI to individuals with TT genotype for rs10783219 (aHR 6.09; 95% CI 1.24 to 30.01); had no effect on individuals with AT genotype (aHR 1.32; 95% CI 0.81 to 2.17), and offered protection to individuals with AA genotype (aHR 0.49; 95% CI 0.29 to 0.85 – aRHR 0.42; 95% CI 0.29 to 0.85; P value for interaction = 0.001). Vitamin D supplementation offered protection from URI to individuals with CT genotype for rs11568820 (aHR 0.51; 95% CI 0.29 to 0.89), but not to individuals with CC genotype (aHR 1.36; 95% CI 0.87 to 2.13 – aRHR 0.35; 95% CI 0.20 to 0.61; P value for interaction <0.001). Vitamin D supplementation offered protection from URI to individuals with CT genotype for rs7976091 (aHR 0.49; 95% CI 0.28 to 0.86), but not to individuals with CC genotype (aHR 1.33; 95% CI 0.85 to 2.08 – aRHR 0.35; 95% CI 0.20 to 0.61; P value for interaction <0.001). Finally, vitamin D supplementation offered protection from URI to individuals with AG genotype for rs7970314 (aHR 0.43; 95% CI 0.25 to 0.74), but not to individuals with AA genotype (aHR 1.27; 95% CI 0.80 to 2.00), or individuals with GG genotype (aHR 0.96; 95% CI 0.14 to 6.65 – aRHR 0.45; 95% CI 0.26 to 0.76; P value for interaction = 0.003).

Multivariable Cox regression analysis also identified 1 SNP which modifies the effect of vitamin D supplementation on risk of COPD exacerbation: Vitamin D supplementation offered protection from exacerbation to individuals with GG genotype for rs2248137 in *CYP24A1* (aHR 0.24; 95 % CI 0.08 to

0.72), but not to individuals with CG genotype (aHR 1.03; 95% CI 0.61 to 1.73), or individuals with CC genotype (aHR 1.04; 95% CI 0.47 to 2.30 – aRHR 0.51; 95% CI 0.29 to 0.91; P value for interaction = 0.023).

Table 9.3. Interaction analysis: Time to first URI.

Gene	SNP	Geno-type	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within sub-group (95% CI) [1]	Ratio of hazard ratios for allocation*genotype interaction (95% CI) [1]	P value for interaction
CUBN	rs3740165	TT	113	154 (27 to UD)	110	203 (33 to UD)	0.93 (0.66 to 1.31)	0.70 (0.18 to 2.77)	0.61
		TC	3*	-	9	226 (147 to UD)	-		
		CC	1*	-	0	-	-		
CYP24A1	rs2762939	GG	61	159 (22 to UD)	72	282 (44 to UD)	0.56 (0.35 to 0.91)	1.73 (1.02 to 2.95)	0.042
		CG	52	143 (32 to 374)	41	123 (33 to 348)	1.40 (0.83 to 2.35)		
		CC	4*	-	7	120 (62 to 344)	-		
	rs2248137	CC	40	176 (26 to 374)	46	226 (27 to 372)	0.94 (0.51 to 1.71)	0.76 (0.46 to 1.23)	0.26
		CG	64	110 (21 to UD)	57	198 (59 to UD)	0.79 (0.50 to 1.25)		
		GG	13	263 (32 to UD)	17	UD (92 to UD)	0.40 (0.12 to 1.35)		
	rs2762934	GG	78	152 (21 to UD)	89	231 (45 to UD)	0.77 (0.51 to 1.15)	1.31 (0.71 to 2.40)	0.38
		AG	39	144 (33 to 374)	26	123 (33 to 300)	1.38 (0.72 to 2.64)		
		AA	1*	-	5	62 (42 to UD)	-		
	rs6013897	TT	73	137 (21 to UD)	83	244 (45 to UD)	0.69 (0.46 to 1.03)	1.59 (0.91 to 2.81)	0.11
		AT	39	217 (41 to UD)	28	147 (27 to UD)	1.62 (0.82 to 3.22)		
		AA	4*	-	8	92 (51 to 239)	-		
CYP27A1	rs17470271	AA	47	156 (33 to 374)	43	217 (54 to UD)	0.74 (0.41 to 1.33)	1.11 (0.71 to 1.74)	0.64
		AT	54	171 (23 to UD)	54	173 (33 to 372)	0.75 (0.45 to 1.23)		
		TT	17	136 (15 to UD)	23	198 (44 to 344)	1.36 (0.57 to 3.21)		
CYP27B1	rs4646537	TT	111	152 (23 to UD)	109	83 (21 to UD)	0.79 (0.56 to 1.18)	2.80 (0.77 to 10.17)	0.12
		GT	7	261 (62 to UD)	10	261 (45 to UD)	2.82 (0.60 to 13.30)		
		GG	0	-	0	-	-		
	rs4646536	AA	48	143 (17 to UD)	47	231 (28 to UD)	0.89 (0.51 to 1.57)	0.97 (0.58 to 1.62)	0.92
		AG	56	152 (27 to 374)	57	203 (50 to UD)	0.79 (0.49 to 1.28)		
		GG	10	144 (9 to UD)	15	147 (18 to UD)	0.86 (0.25 to 2.93)		
CYP2R1	rs10500804	TT	36	108 (27 to UD)	44	147 (31 to 362)	0.97 (0.55 to 1.71)	1.02 (0.65 to 1.61)	0.93
		GT	62	136 (17 to UD)	53	217 (55 to 372)	0.80 (0.48 to 1.34)		
		GG	18	261 (64 to UD)	24	282 (19 to UD)	0.75 (0.30 to 1.89)		
	rs2060793	GG	39	156 (22 to UD)	41	231 (83 to UD)	0.58 (0.32 to 1.07)	1.46 (0.91 to 2.35)	0.12
		AG	58	152 (27 to UD)	45	226 (45 to UD)	0.93 (0.57 to 1.53)		
		AA	20	143 (53 to UD)	22	109 (22 to 327)	2.27 (0.91 to 5.67)		
	rs10766197	GG	31	261 (43 to UD)	36	239 (51 to UD)	1.07 (0.56 to 2.03)	1.03 (0.66 to 1.61)	0.90
		AG	61	110 (21 to UD)	55	216 (50 to 372)	0.78 (0.48 to 1.27)		
		AA	25	261 (43 to UD)	26	178 (27 to UD)	0.91 (0.41 to 2.03)		
CYP3A4	rs2740574	AA	107	154 (23 to UD)	107	200 (42 to UD)	0.86 (0.61 to 1.22)	0.64 (0.20 to 2.02)	0.45
		AG	10	64 (58 to UD)	13	231 (118 to UD)	0.48 (0.14 to 1.65)		
		GG	0	-	0	-	-		
DBP	rs7041	CC	28	202 (23 to UD)	29	226 (50 to 372)	0.99 (0.50 to 1.95)	0.72 (0.45 to 1.16)	0.18
		AC	58	152 (21 to UD)	67	212 (27 to UD)	0.94 (0.59 to 1.48)		
		AA	29	136 (53 to 374)	23	282 (83 to UD)	0.48 (0.21 to 1.08)		
	rs4588	GG	59	197 (41 to UD)	53	244 (50 to UD)	1.04 (0.63 to 1.70)	0.81 (0.49 to 1.31)	0.39
		GT	47	95 (16 to UD)	60	198 (27 to UD)	0.78 (0.48 to 1.29)		
		TT	12	137 (53 to 176)	8	118 (51 to UD)	0.56 (0.13 to 2.45)		
	rs12512631	TT	55	242 (64 to UD)	43	173 (50 to UD)	0.82 (0.48 to 1.38)	1.15 (0.70 to 1.90)	0.58
		CT	51	154 (15 to UD)	63	226 (27 to UD)	0.94 (0.57 to 1.54)		
		CC	11	137 (35 to 374)	12	118 (45 to 344)	1.23 (0.38 to 3.91)		
	rs2070741	TT	99	154 (26 to UD)	106	216 (42 to UD)	0.84 (0.59 to 1.20)	1.08 (0.43 to 2.75)	0.86
		TG	16	108 (43 to UD)	11	198 (54 to UD)	1 .12 (0.31 to 3.99)		
		GG	2*	-	1*	-	-		
	rs2298849	AA	73	159 (28 to UD)	86	231 (44 to UD)	0.80 (0.53 to 1.22)	0.98 (0.54 to 1.78)	0.94
		AG	41	108 (22 to UD)	32	200 (50 to 365)	0.74 (0.41 to 1.36)		
		GG	4*	-	3*	-	-		
	rs16846876	AA	55	156 (27 to UD)	53	226 (34 to UD)	0.98 (0.60 to 1.62)	0.72 (0.44 to 1.18)	0.20
		AT	53	171 (22 to UD)	56	198 (50 to UD)	0.87 (0.53 to 1.42)		
		TT	10	83 (32 to 176)	10	327 (83 to UD)	0.40 (0.06 to 2.52)		

Table 9.3 continued.

Gene	SNP	Geno-type	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within sub-group (95% CI) [1]	Ratio of hazard ratios for allocation*genotype interaction (95% CI) [1]	P value for interaction
DHCR7	rs3829251	GG	90	144 (27 to UD)	91	239 (57 to UD)	0.80 (0.55 to 1.18)	1.42 (0.74 to 2.74)	0.30
		AG	22	171 (32 to UD)	27	54 (19 to UD)	1.13 (0.52 to 2.42)		
		AA	5	27 (22 to UD)	1*	-	-		
	rs12785878	TT	71	144 (33 to UD)	77	217 (44 to UD)	0.91 (0.60 to 1.38)	0.88 (0.51 to 1.52)	0.65
		GT	38	154 (22 to UD)	37	216 (51 to 372)	0.78 (0.43 to 1.41)		
		GG	7	64 (22 to UD)	7	54 (25 to UD)	1.20 (0.17 to 8.32)		
LRP2	rs3755166	GG	39	64 (17 to UD)	43	231 (92 to UD)	0.64 (0.36 to 1.14)	1.47 (0.94 to 2.31)	0.091
		AG	54	171 (27 to 374)	54	212 (42 to 372)	0.92 (0.56 to 1.49)		
		AA	24	242 (32 to UD)	24	118 (27 to UD)	1.28 (0.58 to 2.82)		
RXRA	rs7861779	GG	84	156 (27 to UD)	87	198 (31 to 372)	1.03 (0.70 to 1.51)	0.51 (0.25 to 1.04)	0.063
		GA	29	83 (21 to 346)	25	UD (62 to UD)	0.54 (0.25 to 1.16)		
		AA	4*	-	1*	-	-		
	rs9409929	GG	47	176 (32 to UD)	51	244 (62 to UD)	0.89 (0.51 to 1.56)	1.03 (0.62 to 1.71)	0.92
		AG	58	137 (26 to UD)	62	200 (27 to UD)	0.83 (0.52 to 1.31)		
		AA	12	53 (7 to 242)	6	117 (27 to 300)	0.35 (0.06 to 1.86)		
VDR	rs4334089	GG	64	346 (90 to UD)	57	212 (45 to 365)	1.65 (1.00 to 2.70)	0.36 (0.22 to 0.60)	<0.001†
		AG	42	41 (14 to 154)	55	226 (34 to UD)	0.45 (0.27 to 0.76)		
		AA	12	26 (15 to 171)	8	70 (10 to UD)	0.90 (0.13 to 6.21)		
	rs10783219	TT	16	217 (89 to UD)	8	50 (26 to 212)	6.09 (1.24 to 30.01)	0.42 (0.24 to 0.71)	0.001†
		AT	50	242 (54 to UD)	61	200 (42 to 365)	1.32 (0.81 to 2.17)		
		AA	51	62 (17 to 261)	47	226 (54 to UD)	0.49 (0.29 to 0.85)		
	rs4516035	TT	40	136 (26 to UD)	39	327 (50 to UD)	0.60 (0.33 to 1.11)	1.10 (0.67 to 1.83)	0.70
		CT	62	171 (27 to UD)	65	118 (25 to 372)	1.09 (0.70 to 1.70)		
		CC	14	154 (35 to UD)	16	226 (57 to UD)	0.46 (0.14 to 1.45)		
	rs11568820	CC	70	261 (64 to UD)	68	198 (44 to 372)	1.36 (0.87 to 2.13)	0.35 (0.20 to 0.61)	<0.001†
		CT	39	62 (11 to 374)	48	244 (33 to UD)	0.51 (0.29 to 0.89)		
		TT	7	26 (15 to 47)	4*	-	-		
	rs7976091	CC	71	250 (64 to UD)	68	198 (44 to 372)	1.33 (0.85 to 2.08)	0.35 (0.20 to 0.61)	<0.001†
		CT	39	62 (11 to 374)	49	244 (34 to UD)	0.49 (0.28 to 0.86)		
		TT	8	26 (15 to 47)	4*	-	-		
	rs2238136	CC	63	137 (20 to UD)	58	226 (27 to UD)	0.71 (0.44 to 1.14)	1.23 (0.71 to 2.14)	0.46
		CT	42	242 (58 to UD)	53	198 (83 to 362)	1.27 (0.75 to 2.16)		
		TT	10	89 (17 to UD)	6	UD (25 to UD)	0.37 (0.05 to 2.76)		
	rs1544410	CC	38	159 (28 to UD)	47	231 (42 to UD)	0.65 (0.35 to 1.21)	1.11 (0.69 to 1.79)	0.68
		CT	55	152 (27 to 374)	59	173 (50 to 372)	1.02 (0.63 to 1.63)		
		TT	25	90 (17 to 346)	14	282 (33 to 348)	0.80 (0.34 to 1.88)		
	rs2228570	GG	50	202 (43 to UD)	44	231 (62 to UD)	0.91 (0.52 to 1.58)	1.01 (0.63 to 1.61)	0.97
		AG	48	119 (16 to 374)	60	200 (44 to 372)	0.79 (0.48 to 1.30)		
		AA	19	137 (21 to UD)	16	45 (10 to UD)	0.89 (0.37 to 2.15)		
	rs2853559	GG	42	136 (23 to 374)	37	348 (70 to UD)	0.52 (0.29 to 0.93)	1.26 (0.79 to 2.01)	0.34
		AG	51	209 (28 to UD)	64	120 (28 to 344)	1.32 (0.81 to 2.14)		
		AA	23	176 (47 to UD)	16	231 (57 to UD)	0.59 (0.29 to 1.53)		
	rs7975232	AA	33	143 (27 to UD)	32	120 (33 to UD)	0.87 (0.48 to 1.58)	1.02 (0.64 to 1.63)	0.92
		AC	57	144 (22 to 374)	60	212 (51 to UD)	0.75 (0.46 to 1.21)		
		CC	23	217 (62 to UD)	27	226 (25 to UD)	0.88 (0.38 to 2.05)		
	rs7970314	AA	66	242 (61 to UD)	64	198 (42 to UD)	1.27 (0.80 to 2.00)	0.45 (0.26 to 0.76)	0.003†
		AG	41	95 (14 to 263)	52	244 (44 to UD)	0.43 (0.25 to 0.74)		
		GG	9	27 (23 to 64)	5	UD (5 to UD)	0.96 (0.14 to 6.65)		
	rs731236	AA	40	110 (28 to UD)	49	231 (44 to UD)	0.62 (0.34 to 1.13)	1.18 (0.73 to 1.90)	0.49
		AG	56	154 (22 to 374)	58	173 (34 to 372)	1.05 (0.66 to 1.68)		
		GG	22	90 (23 to UD)	13	344 (45 to UD)	0.78 (0.30 to 1.99)		

[1] *Adjusted for stratification factors i.e. Percent predicted forced expiratory volume in 1 second (<50% vs. ≥50%) and inclusion in vs. exclusion from sputum induction sub-study; significant predictors of upper respiratory infection i.e. Age (<70 years vs. ≥70 years), and smoking status (current vs. non-current); and allocation to study intervention or placebo arm.* ***†*** *Remains significant after correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%.* ******* *Genotype could not be analysed due to <5 participants and/or too few events within this sub category.*

Abbreviations: SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, CI: Confidence interval, UD: Undefined, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor, CUBN: Cubilin.

Table 9.4. Interaction analysis: Time to first exacerbation.

Gene	SNP	Geno-type	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within sub-group (95% CI) [1]	Ratio of hazard ratios for allocation*genotype interaction (95% CI) [1]	P value for interaction
CUBN	rs3740165	TT	113	278 (89 to UD)	110	UD (85 to UD)	0.86 (0.58 to 1.26)	1.69 (0.35 to 8.15)	0.51
		TC	3*	-	9	UD (89 to UD)	-		
		CC	1*	-	0	-	-		
CYP24A1	rs2762939	GG	61	304 (58 to UD)	72	350 (68 to UD)	0.82 (0.49 to 1.38)	0.77 (0.41 to 1.46)	0.42
		CG	52	274 (120 to UD)	41	UD (87 to UD)	0.95 (0.51 to 1.79)		
		CC	4*	-	7	UD (263 to UD)	-		
	rs2248137	CC	40	368 (112 to UD)	46	UD (67 to UD)	1.04 (0.47 to 2.30)	0.51 (0.29 to 0.91)	0.023
		CG	64	342 (120 to UD)	57	329 (108 to UD)	1.03 (0.61 to 1.73)		
		GG	13	64 (22 to 153)	17	UD (72 to UD)	0.24 (0.08 to 0.72)		
	rs2762934	GG	78	371 (89 to UD)	89	350 (87 to UD)	1.02 (0.64 to 1.61)	0.68 (0.35 to 1.34)	0.27
		AG	39	189 (103 to UD)	26	UD (87 to UD)	0.64 (0.29 to 1.39)		
		AA	1*	-	5	263 (163 to UD)	-		
	rs6013897	TT	73	342 (112 to UD)	83	350 (87 to UD)	1.02 (0.65 to 1.60)	0.75 (0.39 to 1.43)	0.38
		AT	39	228 (69 to UD)	28	UD (44 to UD)	0.69 (0.30 to 1.61)		
		AA	4*	-	8	224 (89 to UD)	-		
CYP27A1	rs17470271	AA	47	278 (77 to UD)	43	224 (67 to UD)	1.27 (0.68 to 2.35)	0.69 (0.40 to 1.18)	0.18
		AT	54	261 (112 to UD)	54	UD (177 to UD)	0.55 (0.30 to 0.99)		
		TT	17	UD (90 to UD)	23	UD (87 to UD)	1.30 (0.41 to 4.07)		
CYP27B1	rs4646537	TT	111	329 (89 to UD)	109	351 (87 to UD)	0.91 (0.62 to 1.34)	0.42 (0.09 to 1.86)	0.25
		GT	7	228 (77 to 261)	10	UD (137 to UD)	0.52 (0.10 to 2.78)		
		GG	0	-	0	-	-		
	rs4646536	AA	48	304 (64 to UD)	47	UD (137 to UD)	0.84 (0.44 to 1.58)	1.03 (0.59 to 1.78)	0.93
		AG	56	252 (77 to UD)	57	263 (59 to UD)	0.93 (0.55 to 1.55)		
		GG	10	371 (240 to UD)	15	UD (39 to UD)	0.69 (0.20 to 2.42)		
CYP2R1	rs10500804	TT	36	274 (69 to UD)	44	UD (67 to UD)	0.95 (0.48 to 1.86)	1.05 (0.62 to 1.77)	0.85
		GT	62	278 (77 to UD)	53	UD (87 to UD)	0.72 (0.41 to 1.26)		
		GG	18	368 (228 to UD)	24	315 (118 to UD)	1.46 (0.50 to 4.27)		
	rs2060793	GG	39	304 (112 to UD)	41	UD (137 to UD)	0.71 (0.36 to 1.42)	1.07 (0.64 to 1.79)	0.81
		AG	58	261 (103 to UD)	45	263 (45 to UD)	1.04 (0.60 to 1.81)		
		AA	20	274 (22 to UD)	22	UD (108 to UD)	0.80 (0.27 to 2.34)		
	rs10766197	GG	31	274 (90 to UD)	36	UD (87 to UD)	0.87 (0.41 to 1.84)	1.13 (0.68 to 1.89)	0.64
		AG	61	278 (62 to UD)	55	UD (72 to UD)	0.74 (0.43 to 1.27)		
		AA	25	368 (228 to UD)	26	315 (125 to UD)	1.27 (0.53 to 3.04)		
CYP3A4	rs2740574	AA	107	304 (90 to UD)	107	UD (87 to UD)	0.86 (0.57 to 1.28)	1.41 (0.43 to 4.57)	0.57
		AG	10	274 (58 to UD)	13	301 (44 to UD)	1.84 (0.50 to 6.74)		
		GG	0	-	0	-			
DBP	rs7041	CC	28	252 (129 to UD)	29	315 (85 to UD)	0.99 (0.47 to 2.10)	0.92 (0.53 to 1.60)	0.78
		AC	58	342 (89 to UD)	67	UD (89 to UD)	0.87 (0.51 to 1.47)		
		AA	29	304 (64 to UD)	23	UD (87 to UD)	0.87 (0.37 to 2.01)		
	rs4588	GG	59	329 (90 to UD)	53	350 (67 to UD)	1.08 (0.62 to 1.90)	0.85 (0.47 to 1.52)	0.57
		GT	47	252 (56 to UD)	60	UD (95 to UD)	0.68 (0.39 to 1.19)		
		TT	12	274 (129 to 368)	8	194 (97 to UD)	2.69 (0.41 to 17.39)		
	rs12512631	TT	55	274 (120 to UD)	43	UD (187 to UD)	0.58 (0.30 to 1.13)	1.38 (0.80 to 2.41)	0.25
		CT	51	342 (38 to UD)	63	301 (67 to UD)	1.08 (0.64 to 1.83)		
		CC	11	201 (77 to UD)	12	163 (45 to UD)	0.93 (0.30 to 2.88)		
	rs2070741	TT	99	274 (64 to UD)	106	UD (90 to UD)	0.81 (0.54 to 1.20)	0.62 (0.22 to 1.74)	0.37
		TG	16	UD (153 to UD)	11	UD (32 to UD)	9.91 (1.38 to 71.36)		
		GG	2*	-	1*	-	-		
	rs2298849	AA	73	304 (77 to UD)	86	315 (87 to UD)	0.95 (0.60 to 1.49)	1.01 (0.65 to 1.58)	0.20
		AG	41	345 (123 to UD)	32	UD (89 to UD)	0.70 (0.34 to 1.47)		
		GG	4*	-	3*	-	-		
	rs16846876	AA	55	251 (56 to UD)	53	UD (72 to UD)	0.68 (0.39 to 1.19)	0.94 (0.52 to 1.70)	0.85
		AT	53	369 (144 to UD)	56	301 (87 to UD)	1.28 (0.73 to 2.17)		
		TT	10	132 (103 to UD)	10	UD (UD to UD)	0.95 (0.03 to 28.64)		
DHCR7	rs3829251	GG	90	278 (89 to UD)	91	UD (87 to UD)	0.79 (0.51 to 1.23)	1.42 (0.66 to 3.07)	0.37

Table 9.4 continued.

Gene	SNP	Geno-type	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within sub-group (95% CI) [1]	Ratio of hazard ratios for allocation*genotype interaction (95% CI) [1]	P value for interaction
		AG	22	153 (103 to UD)	27	301 (87 to UD)	0.66 (0.26 to 1.66)		
		AA	5	UD (UD to UD)	1*	-	-		
	rs12785878	TT	71	274 (89 to UD)	77	351 (67 to UD)	0.90 (0.57 to 1.42)	1.08 (0.57 to 2.05)	0.82
		GT	38	261 (90 to UD)	37	UD (177 to UD)	0.55 (0.26 to 1.16)		
		GG	7	UD (21 to UD)	7	89 (28 to UD)	21.73 (1.32 to 356.45)		
LRP2	rs3755166	GG	39	371 (140 to UD)	43	301 (87 to UD)	1.39 (0.72 to 2.69)	0.84 (0.50 to 1.40)	0.50
		AG	54	201 (57 to UD)	54	UD (87 to UD)	0.59 (0.34 to 1.03)		
		AA	24	278 (123 to UD)	24	UD (85 to UD)	0.96 (0.34 to 2.73)		
RXRA	rs7861779	GG	84	369 (112 to UD)	87	315 (87 to UD)	1.08 (0.70 to 1.67)	0.40 (0.17 to 0.94)	0.035
		GA	29	252 (89 to UD)	25	UD (95 to UD)	0.54 (0.21 to 1.37)		
		AA	4*	-	1*	-	-		
	rs9409929	GG	47	371 (112 (to UD)	51	UD (145 to UD)	0.91 (0.48 to 1.71)	0.93 (0.53 to 1.61)	0.94
		AG	58	240 (57 to UD)	62	UD (45 to UD)	0.81 (0.49 to 1.33)		
		AA	12	UD (278 to UD)	6	UD (187 to UD)	0.23 (0.02 to 2.29)		
VDR	rs4334089	GG	64	278 (64 to 369)	57	UD (108 to UD)	0.82 (0.47 to 1.45)	1.16 (0.66 to 2.06)	0.60
		AG	42	228 (49 to UD)	55	315 (72 to UD)	0.82 (0.46 to 1.47)		
		AA	12	371 (103 to UD)	8	329 (22 to UD)	1.94 (0.36 to 10.38)		
	rs10783219	AA	51	219 (49 to UD)	47	UD (90 to UD)	0.64 (0.35 to 1.20)	1.14 (0.63 to 2.08)	0.67
		AT	50	329 (129 to UD)	61	315 (73 to UD)	1.14 (0.66 to 1.95)		
		TT	16	252 (62 to UD)	8	UD (108 to UD)	0.52 (0.07 to 4.21)		
	rs4516035	TT	40	345 (62 to UD)	39	UD (67 to UD)	0.83 (0.40 to 1.69)	0.73 (0.40 to 1.32)	0.30
		CT	62	342 (129 to UD)	65	224 (85 to UD)	1.23 (0.75 to 2.01)		
		CC	14	77 (23 to UD)	16	UD (UD to UD)	0.20 (0.05 to 0.80)		
	rs11568820	CC	70	252 (89 to UD)	68	UD (87 to UD)	0.78 (0.47 to 1.30)	1.29 (0.69 to 2.41)	0.43
		CT	39	345 (123 to UD)	48	315 (85 to UD)	1.07 (0.58 to 1.95)		
		TT	7	278 (57 to UD)	4*	-	-		
	rs7976091	CC	71	252 (89 to UD)	68	UD (87 to UD)	0.80 (0.48 to 1.34)	1.14 (0.61 to 2.12)	0.68
		CT	39	345 (112 to UD)	49	329 (87 to UD)	1.04 (0.57 to 1.92)		
		TT	8	120 (21 to UD)	4*	-	-		
	rs2238136	CC	63	228 (49 to UD)	58	UD (67 to UD)	0.72 (0.43 to 1.19)	1.28 (0.69 to 2.41)	0.44
		CT	42	368 (133 to UD)	53	UD (90 to UD)	1.13 (0.60 to 2.12)		
		TT	10	UD (240 to UD)	6	UD (UD to UD)	0.46 (0.04 to 5.41)		
	rs1544410	CC	38	261 (123 to UD)	47	263 (90 to UD)	0.98 (0.52 to 1.87)	1.23 (0.71 to 2.13)	0.47
		CT	55	274 (64 to UD)	59	UD (87 to UD)	0.67 (0.38 to 1.17)		
		TT	25	345 (89 to UD)	14	187 (45 to 351)	2.51 (0.96 to 6.58)		
	rs2228570	GG	50	252 (64 to UD)	44	UD (87 to UD)	0.64 (0.35 to 1.18)	1.39 (0.81 to 2.41)	0.23
		AG	48	368 (103 to UD)	60	UD(95 to UD)	0.99 (0.55 to 1.80)		
		AA	19	189 (120 to UD)	16	111 (39 to UD)	1.13 (0.43 to 2.99)		
	rs2853559	GG	42	345 (64 to UD)	37	UD (108 to UD)	0.61 (0.31 to 1.21)	1.12 (0.64 to 1.96)	0.68
		AG	51	342 (123 to UD)	64	UD (111 to UD)	1.06 (0.60 to 1.88)		
		AA	23	129 (77 to UD)	16	301 (44 to UD)	0.63 (0.26 to 1.50)		
	rs7975232	AA	33	345 (89 to UD)	32	351 (73 to UD)	0.92 (0.45 to 1.86)	0.95 (0.55 to 1.62)	0.84
		AC	57	278 (69 to UD)	60	UD (67 to UD)	0.76 (0.43 to 1.35)		
		CC	23	369 (132 to UD)	27	UD (108 to UD)	1.03 (0.42 to 2.49)		
	rs7970314	AA	66	261 (89 to UD)	64	UD (87 to UD)	0.84 (0.50 to 1.42)	1.05 (0.57 to 1.94)	0.88
		AG	41	345 (123 to UD)	52	329 (85 to UD)	1.02 (0.56 to 1.85)		
		GG	9	278 (57 to UD)	5	UD (UD to UD)	0.34 (0.02 to 4.70)		
	rs731236	AA	40	261 (123 to UD)	49	301 (90 to UD)	0.88 (0.47 to 1.64)	1.38 (0.79 to 2.42)	0.26
		AG	56	278 (64 to UD)	58	UD (87 to UD)	0.70 (0.40 to 1.24)		
		GG	22	342 (89 to UD)	13	187 (45 to 351)	3.69 (1.31 to 10.38)		

[1] *Adjusted for stratification factors i.e. Percent predicted forced expiratory volume in 1 second (<50% vs. ≥50%) and inclusion in vs. exclusion from sputum induction sub-study; a significant predictor of COPD exacerbation i.e. use of inhaled corticosteroids (yes vs. no); and allocation to study intervention or placebo arm. After correction for multiple comparisons testing, using Benjamini & Hochberg method with a false discovery rate of 5%, none of the p values for interaction remain significant. * Genotype could not be analysed due to <5 participants and/or too few events within this sub category.*

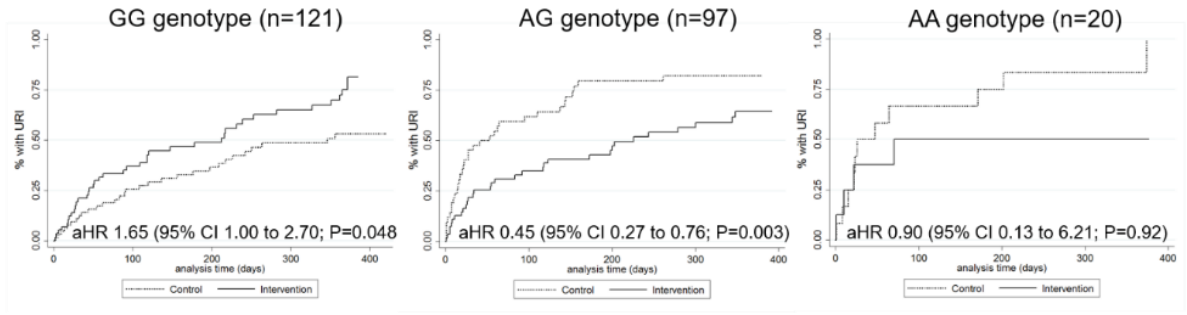
Abbreviations: SNP: Single nucleotide polymorphism, IQR: Inter-quartile range, CI: Confidence interval, UD: Undefined, CYP-: Cytochrome P450 enzyme, DBP: Vitamin D binding protein, DHCR7: 7-dehydrocholesterol reductase enzyme, LRP2: Low density lipoprotein-related protein 2 (also known as megalin), RXRA: Retanoid-X receptor-A, VDR: Vitamin D receptor, CUBN: Cubilin.

In summary, main effects analysis identified five SNP in the VDR gene which show an association with clinical outcomes, independent of vitamin D supplementation: four associate with risk of URI (rs4334089, rs11568820, rs7976091, and rs7970314), and one associates with risk of COPD exacerbation (rs2238136). Interaction analysis identified five SNP in *VDR* which associate with the effect of vitamin D supplementation on risk of URI: rs4334089, rs11568820, rs7976091, rs7970314, rs10783219, and one SNP which associates with the effect of vitamin D supplementation on risk of COPD exacerbation: rs2248137 in *CYP24A1*.

After correction for multiple comparisons testing (Bejamini & Hochberg method with a false discovery rate of 5%) only the interaction analysis findings for rs4334089; rs11568820; rs7976091; rs7970314; rs10783219 and effect of vitamin D supplementation on risk of URI remain significant. Figure 9.1 displays a panel of Kaplan-Meier failure estimates for the effect of allocation on time to first URI event stratified by genotype for each of these 5 SNP, which illustrates the effect modification.

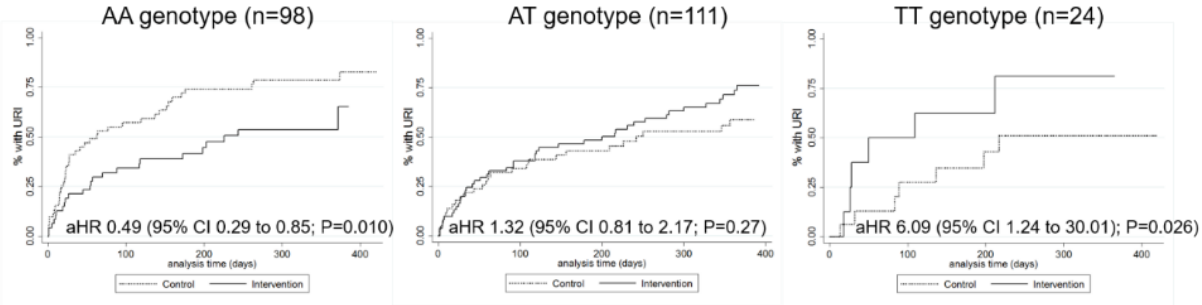
Figure 9.1: Kaplan-Meier failure estimates from the effect modification analysis, illustrating the effect of allocation on number of days post randomisation to first URI event, stratified by genotype for five VDR SNP (rs4334089 [A], rs10783219 [B], rs11568820 [C], rs7970314 [D], and rs7976091 [E]).

A



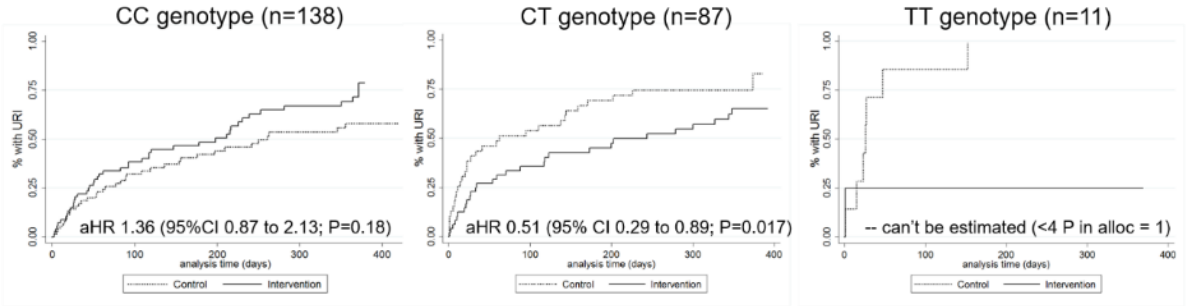
aHR for interaction 0.36 (95% CI 0.22 to 0.60; P<0.001)

B



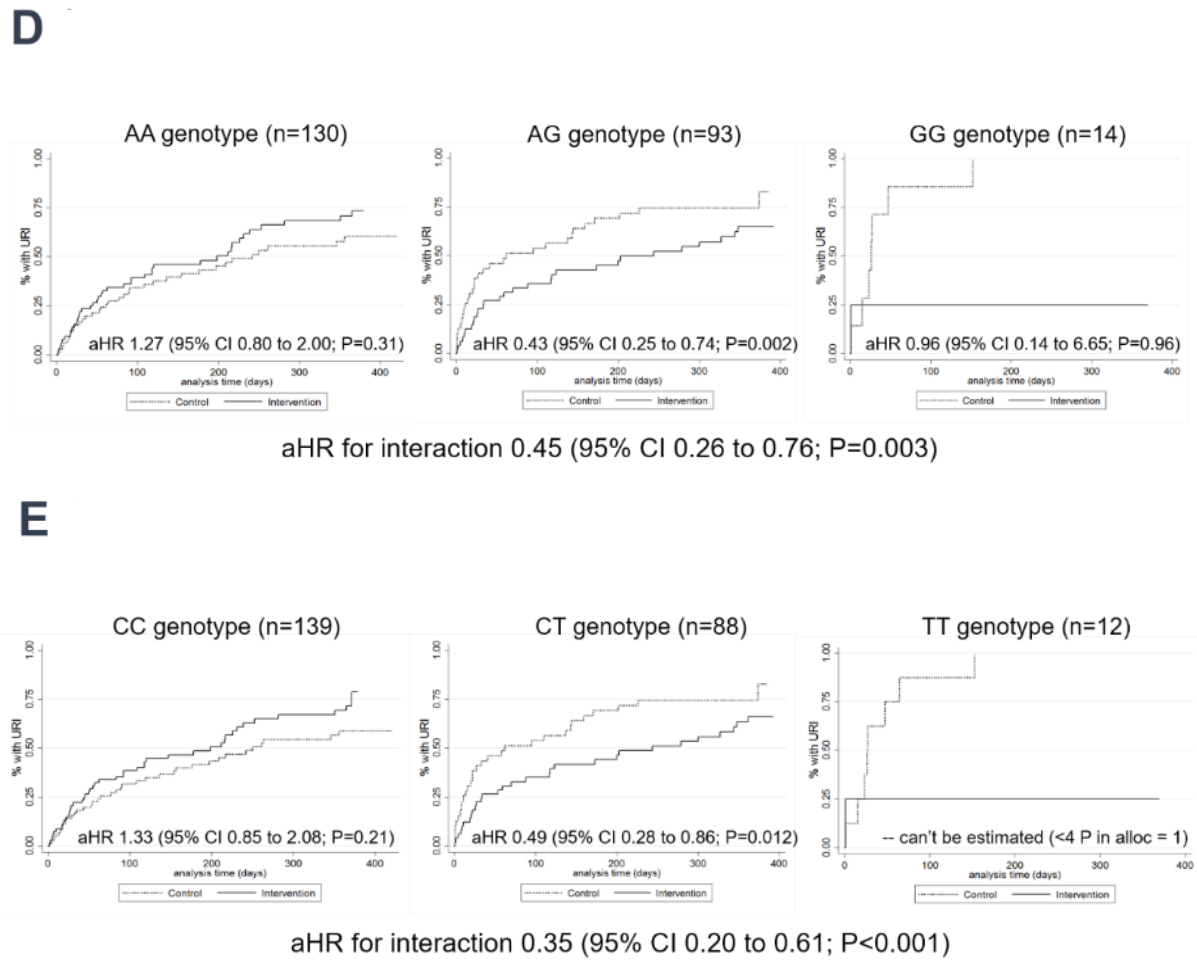
aHR for interaction 3.01 (95% CI 1.53 to 5.94; P=0.001)

C



aHR for interaction 0.35 (95% CI 0.20 to 0.61; P<0.001)

Figure 9.1 continued.



9.2.3. SNP haplotype analysis.

Further investigation of the 5 putative effect modifying *VDR* SNP highlighted a high level of linkage between these variants. Figure 9.2 is a linkage disequilibrium (LD) map generated from the HapMap database (CEU dataset, release #27) which shows that rs4334089 is in perfect LD (R^2 = 1.00) with rs10783219, which in turn is in perfect LD with rs11568820 and rs7970314. LD data were not available in the HapMap database for one SNP (rs7976091) but were available in the 1000 genomes database (integrated phase 1, version 3 [March 2012]), which reported rs7976091 to be tightly linked with rs7970314 (R^2 = 0.90), shown in Table 9.4. Due to this linkage pattern it is reasonable to assign haplotypes which may more closely associate with the effect of vitamin D supplementation on risk of URI than individual variant genotypes do.

Figure 9.2: A linkage disequilibrium map of 4 of the 5 putative effect modifying SNP in VDR gene. Values presented within cells are R^2 measurements of linkage disequilibrium – a value of 1.0 represents perfect linkage, which is represented as red cells. One SNP (rs7976091) was not contained in the HapMap phase 27 dataset.

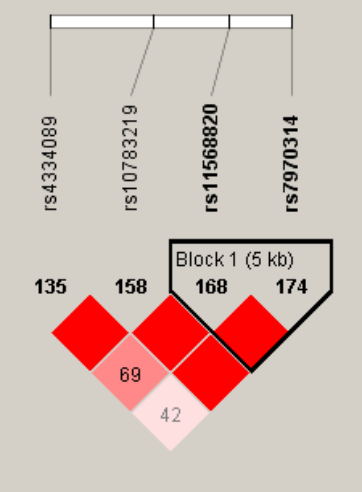


Table 9.5: Linkage disequilibrium between variants rs7976091 and rs7970314.

Variant 1	Variant 2	R^2	D	D prime	Variant 1 (rs#)	Variant 2 (rs#)
chr12:48308174	chr12:48304552	0.902	0.171	0.992	rs7976091	rs7970314

Data retrieved from the 1000 genomes database (integrated phase 1, version 3 [March 2012]).

Using the National Institutes of Health's LDlink tool (http://analysistools.nci.nih.gov) and phased genotype data from the 1000 genomes dataset (Phase 1, version 3 - Utah residents with Northern and Western European ancestry from the CEPH collection [CEU]) six haplotypes were inferred for our 5 *VDR* SNP of interest (presented in Figure 9.3). Manually assigning haplotypes using Clark's method (354) I identified 5 of the 6 most frequent haplotypes in the 1000 genomes dataset to be the most frequently occurring in our participants (GACCA in 75%; GTCCA in 42.9%; AACCA in 40.8%; AATTG in 23.8%; and GATTG in 18.8%).

Figure 9.3: Inferred Haplotypes generated by NIH's LDlink online tools, using 1000 genomes SNP data (Phase 1, Version 3; CEU collection).

SNP	Position (GRCh37)	Allele Frequencies	Haplotypes					
rs4334089	chr12:48286015	G=0.717, A=0.283	G	G	A	A	G	G
rs10783219	chr12:48295488	A=0.636, T=0.364	T	A	A	A	A	A
rs11568820	chr12:48302545	C=0.778, T=0.222	C	C	T	C	C	T
rs7976091	chr12:48304552	C=0.778, T=0.222	C	C	T	C	C	T
rs7970314	chr12:48308174	A=0.737, G=0.263	A	A	G	A	G	G
		Haplotype Count	72	57	39	17	8	5
		Haplotype Frequency	0.3636	0.2879	0.197	0.0859	0.0404	0.0253

Results of the interaction analysis between haplotype and study intervention are presented in Table 9.6 and Figure 9.4. Multivariable Cox regression identified significant independent associations between haplotypes GTCCA, AACCA, and AATTG and the effect of vitamin D supplementation on time to first URI. The strongest protective effect of supplementation was seen in participants with haplotype AACCA (aRHR 0.39; 95% CI 0.23 to 0.67; P=0.001); a milder protective effect was shown in participants with haplotype AATTG (aRHR 0.42; 95% CI 0.21 to 0.84; P=0.013), whilst supplemented participants with haplotype GTCCA had an increased risk of URI (aRHR 2.00; 95% CI 1.17 to 3.41; P=0.011).

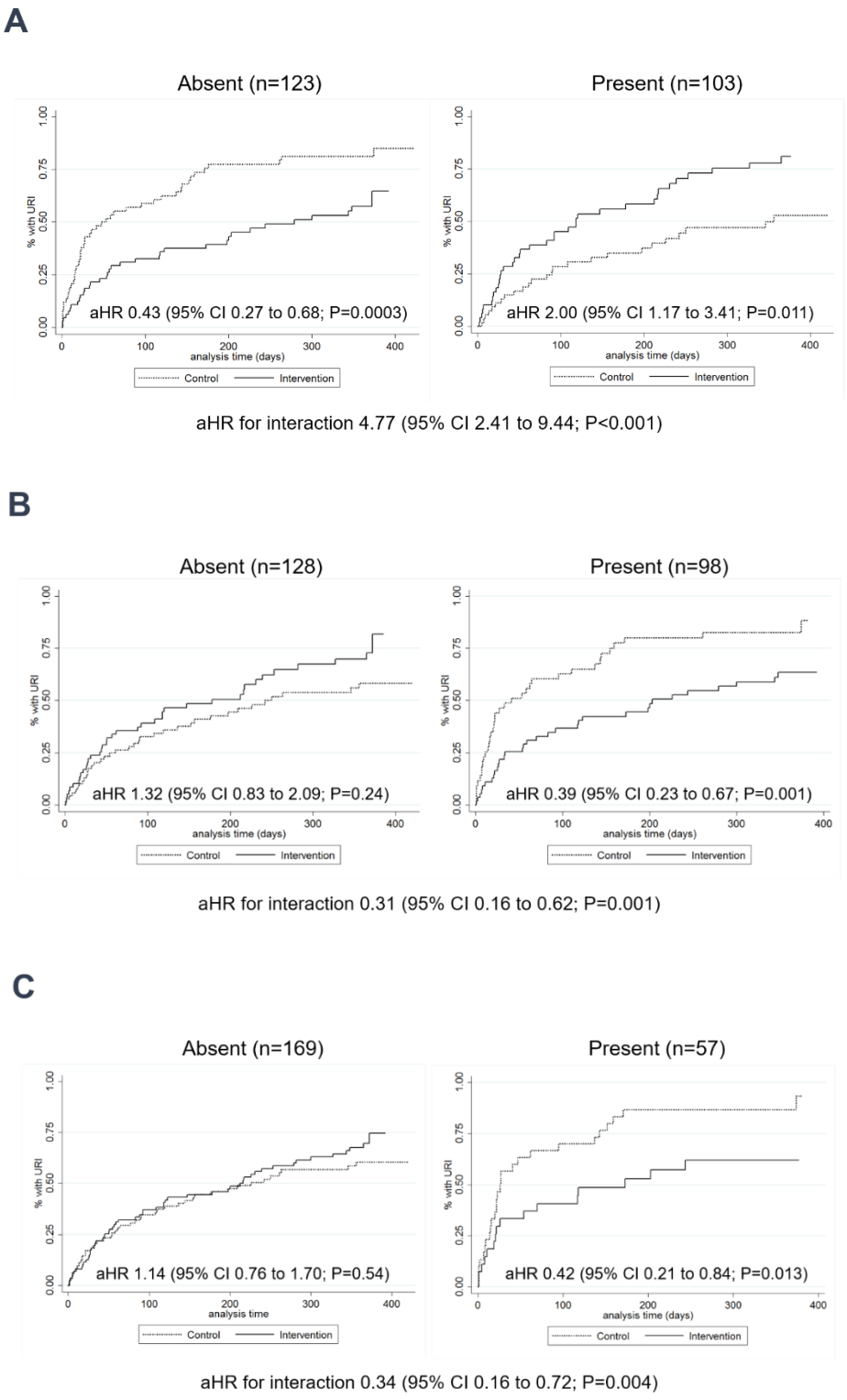
Table 9.6. Haplotype-allocation interaction analysis: Time to first URI.

Haplotype	Positive	N	Median time to 1st URI (IQR) : Control arm	N	Median time to 1st URI (IQR) : Vitamin D arm	Hazard ratio for effect of allocation within sub-group (95% CI) [1]	Ratio of hazard ratios for allocation*haplo-type interaction (95% CI) [1]	P value for interaction
GACCA	1	85	143 (22 to UD)	96	203 (44 to UD)	1.24 (0.51 to 3.03)	1.19 (0.50 to 2.84)	0.69
	0	27	136 (26 to 374)	18	109 (26 to UD)	0.85 (0.58 to 1.23)		
GTCCA	1	54	356 (89 to UD)	49	118 (28 to 282)	2.00 (1.17 to 3.41)	4.77 (2.41 to 9.44)	<0.001
	0	58	47 (15 to 171)	65	279 (55 to UD)	0.43 (0.27 to 0.68)		
AACCA	1	43	41 (11 to 154)	55	203 (34 to UD)	0.39 (0.23 to 0.67)	0.31 (0.16 to 0.62)	0.001
	0	69	242 (61 to UD)	59	178 (42 to 372)	1.32 (0.83 to 2.09)		
AATTG	1	30	26 (14 to 143)	27	173 (21 to UD)	0.42 (0.21 to 0.84)	0.34 (0.16 to 0.72)	0.004
	0	82	226 (54 to UD)	87	212 (45 to UD)	1.14 (0.76 to 1.70)		
GATTG	1	22	27 (14 to 152)	23	173 (21 to UD)	0.46 (0.21 to 1.01)	0.58 (0.26 to 1.29)	0.18
	0	90	176 (33 to UD)	91	212 (44 to UD)	0.97 (0.67 to 1.42)		

[1] *Adjusted for stratification factors i.e. Percent predicted forced expiratory volume in 1 second (<50% vs. ≥50%) and inclusion in vs. exclusion from sputum induction sub-study; significant predictors of upper respiratory infection i.e. Age (<70 years vs. ≥70 years), and smoking status (current vs. non-current); and allocation to study intervention or placebo arm.*

Abbreviations: IQR: Inter-quartile range, CI: Confidence interval.

Figure 9.4: Kaplan-Meier failure estimates illustrating the effect of allocation on number of days post randomisation to first URI event, stratified by three VDR haplotypes: GTCCA (A), AACCA (B), and AATTG (C).



9.2.4. Immune profile analysis.

Based on the strongest signal of effect-modification, I classified participants as genetically defined 'responders' and 'non-responders' to vitamin D supplementation depending on whether they were positive or negative for haplotype AACCA (A allele for rs4334089; A allele for rs10783219; C allele for rs11568820; C allele for rs7976091; A allele for rs7970314). In a subset of n=60 participants I investigated an association between the effects of clinical trial allocation on their inflammatory profile, stratified by the ACCAA haplotype. Participants' inflammatory profile was characterised by measuring the concentration of 30 inflammatory markers (described in methods - chapter.2, section 3.4), in supernatants of whole blood stimulated *ex-vivo* with 5 toll-like receptor (TLR) ligands (lipopolysaccharide [LPS], PAM2, Polyinosinic:polycytidylic acid [Poly I:C], R848, and serum)

Results of the multiple linear regression analysis are presented in Table 9.7. Two trends appeared in these data: lower relative concentrations of Granulocyte-colony stimulating factor (GCSF) were observed for individuals positive for haplotype AACCA receiving vitamin D supplementation, compared to individuals negative for haplotype AACCA receiving vitamin D supplementation, under stimulation with: LPS (GMR 0.79; 95 % CI 0.66 - 0.95; P=0.014 vs. GMR 1.20; 95% CI 1.02 – 1.40; P=0.028. P value for interaction = 0.004); Pam2 (GMR 0.88; 95% CI 0.77 – 1.00; P=0.058 vs. GMR 1.08; 95% CI 0.94 – 1.23; P=0.26. P value for interaction = 0.029); and R848 (GMR 0.88; 95% CI 0.76 – 1.01; P=0.059 vs. GMR 1.04; 95% CI 0.92 – 1.18; P=0.50. P value for interaction = 0.050). Lower relative concentration of Monokine induced by gamma interferon (MIG) were observed for individuals positive for haplotype AACCA receiving vitamin D supplementation, compared to individuals negative for haplotype AACCA receiving vitamin D supplementation, under stimulation with: LPS (GMR 0.98; 95% CI 0.83 - 1.15; P=0.77 vs. GMR 1.30; 95% CI 1.05 – 1.62; P=0.021. P value for interaction = 0.018); Pam2 (GMR 0.96; 95% CI 0.81 – 1.14; P=0.64 vs. GMR 1.35; 95% CI 1.04 – 1.76; P=0.028. P value for trend = 0.026); Poly I:C (GMR 0.89; 95% CI 0.65 – 1.21; P=0.43 vs. GMR 1.33; 95% CI 1.02 – 1.75;

P=0.039. P value for trend = 0.038); and R848 (GMR 0.89; 95% CI 0.64 – 1.23; P=0.46 vs. GMR 1.67; 95% CI 0.17 – 2.37; P=0.006. P value for trend = 0.006).

After correction for multiple comparisons testing (Bejamini & Hochberg method with a false discovery rate of 5%) these associations did not remain significant.

Table 9.7. TLR ligand-stimulated inflammatory cytokine release from PBMCs, stratified by AACCA haplotype (subgroup of n=60).

	Lipopolysaccharide (LPS)					Pam2					Polyinosinic:polycytidylic acid (Poly I:C)				
	effect of allocation : AACCA positive		effect of allocation : AACCA negative		P value for Interaction	effect of allocation : AACCA positive		effect of allocation : AACCA negative		P value for Interaction	effect of allocation : AACCA positive		effect of allocation : AACCA negative		P value for Interaction
Analyte	**GMR (95% CI)**	**P**	**GMR (95% CI)**	**P**		**GMR (95% CI)**	**P**	**GMR (95% CI)**	**P**		**GMR (95% CI)**	**P**	**GMR (95% CI)**	**P**	
FGF Basic	0.99 (0.86 - 1.15)	0.93	1.10 (0.88 – 1.38)	0.40	0.58	0.96 (0.62 – 1.49)	0.87	1.00 (0.63 – 1.58)	1.00	0.94	0.82 (0.54 – 1.25)	0.34	0.87 (0.52 – 1.45)	0.58	0.87
IL-1β	0.74 (0.49 - 1.12)	0.15	1.34 (0.79 – 2.27)	0.27	0.074	0.69 (0.51 – 0.92)	0.015	0.96 (0.63 – 1.47)	0.86	0.31	0.82 (0.40 – 1.67)	0.57	1.36 (0.81 – 2.28)	0.23	0.41
G-CSF	0.79 (0.66 - 0.95)	0.014	1.20 (1.02 – 1.40)	0.028	0.004	0.88 (0.77 – 1.00)	0.058	1.08 (0.94 – 1.23)	0.26	0.029	0.97 (0.77 – 1.21)	0.77	1.13 (0.92 – 1.40)	0.24	0.33
IL-10	0.61 (0.40 - 0.92)	0.021	0.97 (0.60 – 1.57)	0.90	0.17	0.53 (0.32 – 0.90)	0.020	0.87 (0.59 – 1.30)	0.49	0.23	1.07 (0.57 – 1.99)	0.84	1.08 (0.81 – 1.45)	0.60	0.73
IL-13	0.87 (0.75 - 1.03)	0.10	1.07 (0.94 – 1.22)	0.27	0.092	0.84 (0.69 – 1.02)	0.081	1.02 (0.85 – 1.23)	0.80	0.15	0.75 (0.59 – 0.96)	0.023	1.07 (0.87 – 1.33)	0.50	0.088
IL-6	-	-	-	-	-	0.56 (0.34 – 0.92)	0.024	1.09 (0.56 – 2.14)	0.79	0.11	0.39 (0.16 – 0.94)	0.036	1.00 (0.49 – 2.04)	1.00	0.12
IL-12	0.58 (0.33 - 1.03)	0.062	1.21 (0.70 – 2.11)	0.49	0.079	0.89 (0.78 – 1.01)	0.064	0.92 (0.82 – 1.04)	0.19	0.35	1.22 (0.73 – 2.04)	0.44	0.88 (0.74 – 1.04)	0.12	0.51
RANTES	-	-	0.59 (0.11 – 3.11)	0.49	-	0.96 (0.42 – 2.17)	0.91	0.46 (0.12 – 1.70)	0.23	0.42	1.20 (0.43 – 3.34)	0.71	0.78 (0.49 – 1.24)	0.28	0.42
Eotaxin/CCL11	0.76 (0.53 - 1.10)	0.14	0.89 (0.75 – 1.07)	0.20	0.43	0.82 (0.56 – 1.23)	0.34	1.03 (0.81 – 1.32)	0.80	0.40	1.10 (0.80 – 1.51)	0.53	1.12 (0.89 – 1.40)	0.32	0.95
IL-17	0.99 (0.85 - 1.16)	0.92	1.04 (0.96 – 1.13)	0.33	0.89	0.96 (0.83 – 1.11)	0.58	0.98 (0.83 – 1.16)	0.81	0.49	0.94 (0.76 – 1.17)	0.58	1.00 (0.79 – 1.26)	1.00	0.77
MIP-1α	0.63 (0.33 - 1.18)	0.14	1.28 (0.72 – 2.29)	0.38	0.096	0.57 (0.38 – 0.86)	0.010	0.80 (0.42 – 1.55)	0.50	0.39	0.66 (0.28 – 1.58)	0.34	1.36 (0.62 – 2.99)	0.43	0.27
GM-CSF	0.86 (0.67 - 1.10)	0.22	1.01 (0.91 – 1.11)	0.90	0.68	0.89 (0.70 – 1.13)	0.32	0.86 (0.61 – 1.20)	0.37	0.48	0.93 (0.69 – 1.26)	0.63	1.02 (0.92 – 1.13)	0.74	0.46
MIP-1β/CCL4	0.68 (0.36 - 1.30)	0.23	0.95 (0.52 – 1.74)	0.87	0.56	0.70 (0.48 – 1.04)	0.077	0.98 (0.60 – 1.60)	0.92	0.33	0.57 (0.26 – 1.23)	0.14	1.29 (0.73 – 2.28)	0.36	0.076
MCP-1/CCL2	0.79 (0.56 - 1.12)	0.17	1.21 (0.85 – 1.73)	0.27	0.073	0.71 (0.38 – 1.32)	0.27	1.32 (0.79 – 2.23)	0.28	0.12	0.99 (0.44 – 2.23)	0.98	1.28 (0.644 – 2.53)	0.47	0.49
IL-15	0.88 (0.71 - 1.10)	0.25	1.20 (0.98 – 1.47)	0.075	0.15	0.85 (0.69 – 1.05)	0.12	1.24 (0.84 – 1.84)	0.27	0.092	0.60 (0.32 – 1.11)	0.099	1.33 (0.72 – 2.43)	0.35	0.071
EGF	0.89 (0.69 - 1.15)	0.37	0.94 (0.81 – 1.09)	0.39	0.93	0.98 (0.67 – 1.44)	0.92	0.80 (0.65 – 1.00)	0.046	0.31	0.86 (0.66 – 1.13)	0.26	0.93 (0.78 – 1.12)	0.44	0.93
IL-5	0.95 (0.87 - 1.04)	0.26	1.07 (0.96 – 1.19)	0.21	0.17	0.95 (0.87 – 1.05)	0.33	1.07 (0.96 – 1.19)	0.19	0.18	0.99 (0.89 – 1.10)	0.85	1.03 (0.92 – 1.15)	0.63	0.53
HGF	0.91 (0.83 - 1.01)	0.063	1.09 (0.96 – 1.22)	0.17	0.021	0.91 (0.77 – 1.06)	0.21	0.98 (0.83 – 1.16)	0.83	0.43	0.91 (0.75 – 1.11)	0.34	1.03 (0.86 – 1.25)	0.72	0.35
VEGF	0.96 (0.83 - 1.10)	0.52	1.18 (0.99 – 1.41)	0.068	0.17	0.80 (0.67 – 0.95)	0.015	0.97 (0.77 – 1.22)	0.81	0.18	0.77 (0.51 – 1.16)	0.20	1.12 (0.84 – 1.49)	0.44	0.13
IFN-γ	0.91 (0.60 - 1.38)	0.66	1.36 (1.03 – 1.78)	0.029	0.083	1.02 (0.93 – 1.12)	0.65	1.05 (0.97 – 1.14)	0.24	0.64	0.98 (0.69 – 1.38)	0.89	0.89 (0.57 – 1.40)	0.61	0.76
IFN-α	0.91 (0.82 - 1.00)	0.052	1.06 (0.98 – 1.15)	0.15	0.033	0.85 (0.70 – 1.04)	0.11	0.96 (0.78 – 1.18)	0.69	0.34	0.95 (0.68 – 1.32)	0.75	0.93 (0.73 – 1.19)	0.56	0.67
IL-1RA	0.84 (0.55 - 1.29)	0.41	1.23 (0.91 – 1.65)	0.17	0.25	0.77 (0.54 – 1.08)	0.13	0.86 (0.56 – 1.32)	0.47	0.75	0.78 (0.51 – 1.19)	0.23	0.85 (0.52 – 1.40)	0.51	0.73
TNF-α	0.76 (0.47 - 1.23)	0.25	1.52 (0.92 – 2.51)	0.10	0.045	0.77 (0.61 – 0.97)	0.028	0.98 (0.56 – 1.70)	0.94	0.42	0.91 (0.54 – 1.56)	0.73	1.29 (0.95 – 1.75)	0.097	0.19
IL-2	0.86 (0.65 - 1.14)	0.29	0.92 (0.73 – 1.16)	0.47	0.87	-	-	-	-	-	-	-	-	-	-
IL-7	0.96 (0.86 - 1.07)	0.44	1.15 (1.01 – 1.32)	0.035	0.13	0.86 (0.66 – 1.13)	0.27	0.89 (0.65 – 1.21)	0.43	0.96	0.61 (0.40 – 0.93)	0.025	1.29 (0.77 – 2.16)	0.33	0.018
IP-10	1.09 (0.57 - 2.10)	0.78	2.77 (1.47 – 5.23)	0.003	0.032	0.95 (0.71 – 1.28)	0.75	1.27 (0.89 – 1.80)	0.18	0.25	1.32 (0.47 – 3.72)	0.58	1.19 (0.41 – 3.46)	0.74	0.94
IL-2R	0.97 (0.87 - 1.08)	0.57	1.17 (1.01 – 1.36)	0.035	0.040	0.97 (0.81 – 1.17)	0.77	1.10 (0.94 – 1.29)	0.24	0.32	1.01 (0.82 – 1.25)	0.92	1.05 (0.84 – 1.32)	0.63	0.73
MIG/CXCL9	0.98 (0.83 - 1.15)	0.77	1.30 (1.05 – 1.62)	0.021	0.018	0.96 (0.81 – 1.14)	0.64	1.35 (1.04 – 1.76)	0.028	0.026	0.89 (0.65 – 1.21)	0.43	1.33 (1.02 – 1.75)	0.039	0.038
IL-4	0.94 (0.81 - 1.09)	0.40	1.09 (0.99 – 1.21)	0.072	0.079	0.90 (0.74 – 1.11)	0.31	0.95 (0.82 – 1.10)	0.45	0.69	0.84 (0.62 – 1.13)	0.23	0.99 (0.85 – 1.15)	0.89	0.30
IL-8	0.85 (0.47 - 1.53)	0.56	1.12 (0.67 – 1.87)	0.65	0.69	0.49 (0.25 – 0.95)	0.036	0.79 (0.43 – 1.44)	0.43	0.29	0.66 (0.25 – 1.77)	0.39	1.04 (0.43 – 2.47)	0.93	0.50

Table 9.7 continued.

	R848					Serum				
	effect of allocation : AACCA positive		effect of allocation : AACCA negative		P value for Interaction	effect of allocation : AACCA positive		effect of allocation : AACCA negative		P value for Interaction
Analyte	**GMR (95% CI)**	**P**	**GMR (95% CI)**	**P**		**GMR (95% CI)**	**P**	**GMR (95% CI)**	**P**	
FGF Basic	0.98 (0.81 – 1.18)	0.81	1.10 (0.92 – 1.31)	0.28	0.37	-	-	-	-	-
IL-1β	0.81 (0.53 – 1.25)	0.33	1.27 (0.89 – 1.82)	0.18	0.11	-	-	-	-	-
G-CSF	0.88 (0.76 – 1.01)	0.059	1.04 (0.92 – 1.18)	0.50	0.050	1.11 (0.79 – 1.57)	0.53	0.97 (0.77 – 1.22)	0.78	0.55
IL-10	0.79 (0.51 – 1.23)	0.28	1.03 (0.76 – 1.40)	0.82	0.20	1.13 (0.70 – 1.82)	0.59	1.16 (0.95 – 1.42)	0.15	0.56
IL-13	0.90 (0.79 – 1.04)	0.14	1.05 (0.91 – 1.22)	0.48	0.15	0.93 (0.56 – 1.56)	0.79	0.98 (0.65 – 1.47)	0.91	0.94
IL-6	-	-	-	-	-	1.18 (0.84 – 1.66)	0.32	1.08 (0.82 – 1.43)	0.55	0.15
IL-12	0.61 (0.37 – 1.00)	0.049	1.14 (0.71 – 1.82)	0.59	0.080	1.02 (0.89 – 1.18)	0.75	1.02 (0.87 – 1.19)	0.81	0.89
RANTES	1.23 (0.52 – 2.87)	0.60	0.47 (0.09 – 2.50)	0.34	0.29	0.92 (0.43 – 1.99)	0.82	0.75 (0.45 – 1.26)	0.26	0.66
Eotaxin/CCL11	1.02 (0.84 – 1.25)	0.83	0.97 (0.81 – 1.15)	0.68	0.66	1.03 (0.79 – 1.34)	0.84	1.06 (0.83 – 1.35)	0.63	0.82
IL-17	0.93 (0.85 – 1.01)	0.071	1.04 (0.94 – 1.14)	0.43	0.062	-	-	-	-	-
MIP-1α	1.22 (0.56 – 2.63)	0.60	1.60 (1.01 – 2.53)	0.048	0.54	1.20 (0.86 – 1.67)	0.27	1.20 (0.97 – 1.47)	0.085	0.29
GM-CSF	0.85 (0.67 – 1.07)	0.16	1.08 (0.91 – 1.27)	0.36	0.81	0.91 (0.70 – 1.19)	0.48	1.13 (0.98 – 1.31)	0.085	0.81
MIP-1β/CCL4	1.68 (0.82 – 3.41)	0.14	1.65 (0.89 – 3.05)	0.10	0.93	1.32 (0.96 – 1.81)	0.089	1.14 (0.88 – 1.47)	0.31	0.19
MCP-1/CCL2	1.06 (0.72 – 1.56)	0.75	1.19 (0.95 – 1.49)	0.12	0.59	1.12 (0.85 – 1.47)	0.41	1.22 (0.94 – 1.58)	0.13	0.70
IL-15	0.97 (0.72 – 1.30)	0.82	1.04 (0.94 – 1.16)	0.44	0.58	-	-	-	-	-
EGF	0.88 (0.72 – 1.06)	0.17	0.97 (0.87 – 1.08)	0.58	0.48	1.65 (0.95 – 2.88)	0.073	0.79 (0.43 – 1.46)	0.44	0.10
IL-5	0.99 (0.92 – 1.08)	0.86	1.03 (0.92 – 1.15)	0.59	0.67	1.11 (1.01 – 1.21)	0.025	1.06 (0.96 – 1.18)	0.22	0.59
HGF	0.94 (0.83 – 1.07)	0.37	1.06 (0.93 – 1.20)	0.36	0.19	1.51 (1.13 – 2.01)	0.007	1.06 (0.88 – 1.27)	0.53	0.019
VEGF	0.99 (0.80 – 1.23)	0.93	1.06 (0.87 – 1.29)	0.54	0.60	-	-	-	-	-
IFN-γ	0.66 (0.26 – 1.66)	0.37	2.30 (1.21 – 4.39)	0.013	0.016	1.01 (0.96 – 1.06)	0.77	1.06 (1.00 – 1.12)	0.036	0.16
IFN-α	0.70 (0.50 – 0.96)	0.030	0.93 (0.66 – 1.30)	0.64	0.16	1.06 (0.92 – 1.23)	0.42	1.09 (0.95 – 1.25)	0.21	1.00
IL-1RA	0.80 (0.53 – 1.22)	0.29	0.77 (0.42 – 1.43)	0.40	0.98	1.17 (0.84 – 1.63)	0.33	1.34 (0.98 – 1.83)	0.065	0.58
TNF-α	0.66 (0.38 – 1.15)	0.13	1.67 (1.06 – 2.61)	0.027	0.012	1.27 (0.88 – 1.83)	0.20	1.08 (0.77 – 1.51)	0.65	0.45
IL-2	0.92 (0.78 – 1.08)	0.28	1.04 (0.91 – 1.20)	0.54	0.30	-	-	-	-	-
IL-7	0.94 (0.82 – 1.08)	0.37	1.09 (0.93 – 1.27)	0.27	0.34	-	-	-	-	-
IP-10	-	-	-	-	-	1.03 (0.85 – 1.24)	0.76	1.13 (0.91 – 1.41)	0.26	0.50
IL-2R	1.02 (0.87 – 1.19)	0.84	1.11 (0.98 – 1.26)	0.086	0.33	1.18 (0.94 – 1.48)	0.15	1.15 (0.95 – 1.39)	0.14	0.86
MIG/CXCL9	0.89 (0.64 – 1.23)	0.46	1.67 (0.17 – 2.37)	0.006	0.006	1.17 (0.93 – 1.48)	0.17	1.41 (1.02 – 1.94)	0.037	0.37
IL-4	0.97 (0.85 – 1.11)	0.67	1.05 (0.95 – 1.17)	0.32	0.34	1.03 (0.87 – 1.21)	0.75	1.12 (0.93 – 1.34)	0.22	0.46
IL-8	0.66 (0.41 – 1.06)	0.085	0.79 (0.45 – 1.39)	0.40	0.69	0.95 (0.46 – 1.97)	0.88	1.03 (0.54 – 1.96)	0.93	0.82

** None of the p values were significant after correcting for multiple comparison testing using the Benjamini & Hochberg method with a false discovery rate of 5%.*

*** A small constant (0.05) was added to each analytes' median concentration before log transforming for the regression analysis, to avoid taking logs of 0.*

Abbreviations: GMR: Geometric mean ratio. FGF Basic: Fibroblast growth factor basic; GCSF: Granulocyte-colony stimulating factor; IL-n: Interleukin-n; RANTES: Regulated on Activation, Normal T Cell Expressed and Secreted; MIP-1α: Macrophage inflammatory protein 1 alpha; GMCSF: Granulocyte macrophage colony-stimulating factor; MIP-1β: Macrophage inflammatory protein 1 beta; Monocyte chemoattractant protein 1; EFG: epidermal growth factor; HPG: Hepatocyte growth factor; VEGF: Vascular endothelial growth factor; IFN-γ: Interferon gamma; IFN-α: Interferon alpha; IL-1RA: Interleukin-1 receptor antagonist; TNF-α: Tumour necrosis factor alpha; IP-10: IFN-γ-Inducible protein 10; IL-2R: Interleukin-2 receptor; MIG: Monokine induced by gamma interferon.

9.2.5. VDR expression analysis.

The enhanced response to vitamin D supplementation I observed in AACCA positive individuals could be mediated by *VDR* expression i.e. higher expression would result in more receptor available to ligate $1,25(OH)_2D$. One of the 5 putative effect modifying *VDR* SNP, rs11568820, is a binding site for a transcription factor (caudal-type homeobox protein 2 [cdx2]) which has been reported to affect *VDR* expression level (355). To investigate an association between all AACCA haplotype SNP and VDR gene expression level, I carried out reverse transcription-quantitative polymerase chain reaction (RT-qPCR, as described in methods – chapter 2, section 3.3) on whole blood samples in a subgroup of 55 participants. Relative quantification of *VDR* messenger-RNA (mRNA) was calculated using the comparative $2^{-\Delta\Delta CT}$ method (356). Results of this analysis are presented in Table 9.8. Relative to the housekeeping gene (Glyceraldehyde-3-Phosphate Dehydrogenase [GAPDH]), the ratio of *VDR* expression did not significantly differ between individuals with the common genotype vs. heterozygous/uncommon genotypes for any of the 5 SNP comprising the AACCA haplotype.

Table 9.8. RT-qPCR: VDR mRNA expression levels stratified by VDR SNP (subgroup of n=55).

SNP	Average ΔC_t [1]: Common genotype	Average ΔC_t: Heterozygous / uncommon genotype	$\Delta\Delta C_t$	Fold change in VDR expression ($2^{-\Delta\Delta Ct}$)	P value [2]
rs10783219	5.31	5.16	-0.15	1.11	0.79
rs4334089	4.97	5.42	0.45	0.73	0.46
rs11568820	4.90	5.51	0.61	0.66	0.27
rs7976091	4.83	5.56	0.73	0.60	0.17
rs7970314	4.98	5.41	0.44	0.74	0.34

[1] *VDR mRNA cycle threshold (C_t) values were normalised to housekeeping gene (Glyceraldehyde-3-Phosphate Dehydrogenase [GAPDH]) C_t values.* ***[2]*** *Significance of expression fold change was obtained by Mann-Whitney U test of (VDR-C_t)-(GAPDH-C_t) values for those with the common genotype vs. those with heterozygous/uncommon genotype.*

Abbreviations: SNP: Single nucleotide polymorphism, C_t: Cycle threshold, VDR: Vitamin D receptor.

9.3. Discussion.

In this chapter I have investigated the effect of known variants in common vitamin D pathway genes on the risk of URI or disease exacerbation in patients with COPD. By main effects analysis these variants do not significantly associate with either outcome, independent of the effect of vitamin D supplementation. However, I did observe a signal of effect modification in haplotypes of 5 SNP in the vitamin D receptor gene (rs4334089; rs10783219; rs11568820; rs7976091; and rs7970314), the clearest of which was in participants positive for the assigned haplotype AACCA (98/226; 43.4%), in whom vitamin D supplementation conferred a 61% lower risk of URI as compared to those receiving placebo (aHR 0.39; 95% CI 0.23 to 0.67), but in individuals who were negative for AACCA, the intervention had no effect (aHR 1.32; 95% CI 0.83 to 2.09 – aRHR 0.31; 95% CI 0.16 to 0.62; P value for interaction = 0.001).

Several findings relating to non-skeletal disease outcomes have been reported for some of the AACCA haplotype SNP, all of which share an inflammatory basis: rs11568820 and rs7976091 have been linked to risk of late-onset Alzheimer's disease (357); rs11568820 has been found to associate with risk of gout (358); rs7970314 and rs4334089 have been reported to associate with risk of Parkinson's disease (359, 360); finally, rs11568820 and rs7970314 have been linked to TNF-α levels post-rubella vaccination (350). Interestingly, I observed an association between AACCA haplotype and TNF-α concentration released from PBMCs stimulated by LPS and R848 (Table 9.7), though this was prior to correction for multiple comparison testing thus they may be due to type I error.

Quantitative PCR did not reveal a significantly different mean concentration of VDR mRNA by genotype for our five VDR SNP of interest. This suggests these variants do not effect gene expression, which is supported by the lack of previous functional findings for the intronic SNP: rs7976091, rs4334089, rs10783219, or rs7970314. However, rs11568820 is a promoter region SNP located in the binding site for a VDR transcription factor (Cdx2) (336) and electronic mobility shift assay (EMSA) and transfection

experiments have been conducted which show the A allele confers 30% higher VDR promoter transcriptional activity, compared to the G allele (324). If one or more SNP within AACCA haplotype do have a causal effect on vitamin D supplementation in prevention of URI, rs11568820 would seem the most likely candidate, but considering the high degree of linkage between these and neighbouring variants it is not possible to say whether this is the causal allele, without conducting gene sequencing experiments.

The immunological data presented in this chapter do suggest a mechanism by which variation in *VDR* may influence the effect of vitamin D supplementation on risk of URI. Prior to correction for multiple comparison testing, results for AACCA-positive individuals who received vitamin D supplementation display a trend for decreased release of G-CSF (granulocyte colony stimulating factor) and MIG (Monokine induced by γ-interferon/CXCL9) under stimulation from LPS, Pam2, Poly I:C, and R848 in response to supplementation. G-CSF is a growth factor which has been shown to stimulate a rise in monocyte, lymphocyte and neutrophil levels in peripheral blood (361), and has been linked to the exacerbation of inflammatory conditions in patients with rheumatoid arthritis (362, 363), whilst MIG is a chemoattractant chemokine which has been reported to increase T cell and monocyte recruitment to the lung of COPD and tuberculosis patients (364, 365). A common histopathological feature of COPD is elevated neutrophil levels that become further elevated in the face of microbial infection and can result in hyper-inflammatory airway conditions; tissue necrosis, degranulation and lung destruction (94), but a certain level of airway inflammation is required for an effective immune response, thus a reduction in pro-inflammatory leukocyte chemotaxis mediated by a vitamin D-driven reduction in the activation of inflammatory mediators may be responsible for the enhanced protection I have seen in AACCA positive individuals.

9.3.1. Study strengths.

To my knowledge, this is the first study to comprehensively investigate genetic variation in the vitamin D pathway on risk of upper respiratory infection and disease exacerbation in COPD patients participating in a clinical trial of vitamin D supplementation (210). The study population comprised patients with a wide range of disease severity, who were recruited from different hospital and community settings, making them generalisable to larger populations. The majority of participants were deficient at baseline and with intermittent bolus dosing a high level of compliance was achieved, which translated to a good level of repletion in the active arm. Participants were well characterised for possible environmental determinants of ARI, disease exacerbations, and vitamin D status, which allowed for comprehensive control of factors which might confound the association between genetic variation on the outcomes investigated. One final strength, was the use of multiplex ELISA and RT-qPCR to elucidate a mechanism which may explain the findings of genetic association.

9.3.2. Study Limitations.

This study was not without limitation. The overall study population size was powered for the effect of intervention on clinical end-point in all-comers, rather than to detect a signal between genotype and COPD health outcomes, which likely affected the chance of detecting an association between SNP with small minor allele frequencies and COPD exacerbations the most as they were less frequent than URI, but type II error may have been an issue for the more common alleles and outcomes also. However, despite this limitation the investigation did highlight a strong signal between a group of *VDR* SNP and the effects of supplementation on risk of URI, which survived correction for multiple comparison testing, using a strict false discovery rate of 5%. Furthermore, it was only possible to carry out multiplex ELISA and RT-qPCR on a subset of participants, which gave me a relatively small window of observation into the relationship between AACCA haplotype and the effects of supplementation on URI risk. Also it may be that using peripheral blood samples for RT-qPCR was not informative as changes in *VDR* expression may be lung tissue-specific

9.4. Conclusions.

Current clinical trials of vitamin D supplementation in the prevention of URI and exacerbations of COPD (210, 272) have not demonstrated a protective effect from this intervention in their study populations as a whole. However, this analysis has identified a haplotype of 5 *VDR* SNP, possession of which associates with enhanced response to vitamin D supplementation and significantly reduced risk of upper respiratory infection. As URI are a major precipitant of life-threatening exacerbations for this disease that by 2010 had become the 3rd most common cause of death worldwide (366), I believe further investigation of this haplotype is warranted, specifically with a view to elucidating a causal allele, which if found could allow for targeted vitamin D therapy.

10. Concluding remarks and future directions for research.

My thesis has reviewed previous clinical studies which have investigated the potential of vitamin D as an intervention for prevention of ARI; it has investigated the environmental and genetic determinants of vitamin D status in 3 distinct study populations in whom ARI takes a high toll; it has investigated an association between vitamin D deficiency and the clinical features of asthma and COPD, including an investigation of the role of genetic variation; and finally, it has covered the effect of genetic variation on risk of ARI and disease exacerbations, which included an investigation of genetic effect modification of vitamin D supplementation.

Does vitamin D offer protection against ARI?

Chapter 2 presents a systematic review I conducted in the area of vitamin D supplementation in prevention of ARI, which covered clinical studies conducted up until late 2012. The overarching message was of a 'disconnect' that exists between observational studies which consistently report an inverse association between vitamin D metabolite concentrations and risk of ARI, and clinical trials which report highly conflicting outcomes – only half of the 14 published trials at that time reported protective effects from a vitamin D intervention. Since 2012, 13 new trials have been published and a further 2 have completed and are pending publication (these are presented in Table 10.1). ARI was the primary or co-primary outcome in 12 of the 13 published trials, while one investigated a measure of asthma treatment failure (300). Six trials report that vitamin D supplementation offers protection from ARI – 3 in the whole study population (367-369), 1 in a subgroup with profound vitamin D deficiency at baseline (370), one in only the low-dose intervention group (371), and one reports protection only at the mid-point of follow-up (372). One trial reports vitamin D supplementation offered protection from COPD exacerbations in a subgroup of profoundly deficient participants (210). Five trials report null effects of vitamin D supplementation on all respiratory outcomes investigated (209, 300, 373-375), and one trial reports negative effects of vitamin D supplementation on risk of ARI (313). The proportion

of positive reports is comparable to the 14 clinical trials reviewed in Chapter 3 – vitamin D offers protection against ARI in roughly half of the studies. The possible cause of this heterogeneity is less obvious in these more recent trials however because their reports do not align with the major limitations which aligned with the null/negative outcomes in the previous trials i.e. inadequate dosing regimens to attain sustained repletion of serum 25(OH)D concentration in the intervention arm; possible type I or type II error from post-hoc analysis, or prospective analysis of ARI as secondary outcome; and small proportions of vitamin D deficient participants at baseline. Two trends do present themselves however: the majority of positive findings come from trials which administered a daily or weekly dosing regimen (6 of the 7 positive reports (367-372)), whilst only 1 of the 4 trials which administered a monthly bolus dosing regimen reported a positive finding, and this came from a pre-specified analysis of those who were vitamin D deficient (<50 nmol/L) at baseline (210). This trend lends support to the theory that large intermittent bolus doses of vitamin D cause a sharp increase in metabolite concentrations which may trigger a strong negative feedback response with abnormally low 1α-hydroxylase activity and abnormally high 24-hydroxylase activity resulting in lowered extra-renal $1,25(OH)_2D$ concentrations (345). To add further credence to this observation, the preliminary results of our currently unpublished IPD meta-analysis - which includes data from all but one of the trials presented in Table 10.1 (300); the two as yet unpublished trials from Mezawa et al. and Ginde et al.; and 10 of the trials I review in Chapter 3 (268, 269, 271, 272, 274-276, 278-280) – also indicate a significant trial-level effect for protection from ARI in those who received a daily or weekly dosing regimen, but not those who received a monthly or two-monthly bolus dosing regimen. This is a finding that has been previously highlighted in the meta-analysis of some of these trials, albeit with limitations of possible publication bias and a large degree of study heterogeneity (376). Another trend which appears in the trials presented in Table 10.1, and those reviewed in Chapter 3, are signs of greater protection in deficient participants at baseline. Several studies have found no effect of intervention in the entire study group, but have reported protection in subgroups with baseline 25(OH)D levels <25 nmol/L (272), <40 nmol/L (370), and <50 nmol/L (210). One trial was conducted in Mongolia (271) - a

setting that has very few dietary sources of vitamin D and is at a latitude that receives little effective UVR exposure to stimulate cutaneous vitamin D synthesis (377). As a result, their intervention arm had a median baseline 25(OH)D status of just 17.5 nmol/L, and they reported a large reduction in ARI rates (RR 0.50; 95% CI 0.28 – 0.88) from a modest dosing regimen (300 IU/day, for 7 weeks). More trials which report protection from administering effective dosing regimens to deficient study populations are needed before vitamin D can be said to be effective in preventing ARI, but conducting such trials are difficult owed to the ethical issue of randomising deficient participants to the placebo arm. At least one trial has registered to address this need (PRECOVID trial - NCT02122627). The group plans to give a 16,000 IU/week regimen to adult COPD patients with a 25(OH)D concentration between 15-50 nmol/L. IPD meta-analysis of existing trials is the next best thing as it affords us greater statistical power when combining deficient participants across multiple trials. Our IPD meta-analysis supports previous findings: our preliminary analysis shows vitamin D offered a 44% reduction in risk of ARI in those with a baseline vitamin D status of <25 nmol/L (OR 0.56; 95% CI 0.41 to 0.77; P<0.001), compared to a non-significant effect in those with a baseline vitamin D status ≥25 nmol/L (OR 0.90; 0.76 to 1.05; P=0.17 – P value for interaction = 0.004).

The determinants of vitamin D deficiency and features of asthma/COPD phenotype.

Given that evidence from clinical studies highlights populations with serum 25(OH)D concentrations <50 nmol/L as a key target to receive daily dosing regimens of vitamin D for prevention of ARI, the characterisation of factors responsible for vitamin D deficiency in those which suffer the greatest consequences of ARI is all the more imperative. In Chapters Four, Five, and Six I identified a range of classical environmental factors - known to impact vitamin D status in healthy populations - which operate in our 'at-risk' study populations. Use of these findings could lead to a low-cost and highly effective method of correcting vitamin D deficiency in specific patient groups. If these findings are accurate, their impact on vitamin D status is significant. To use the asthma cohort as an example: an adult Londoner who visits their GP for a check-up during winter who does not take a vitamin D

supplement, is unemployed, has not recently taken a sunny holiday abroad, and has a BMI of 25 kg/m^2 or more, would be expected to have a serum 25(OH)D concentration approximately 70 nmol/L lower than if they visited during summer and are taking a daily vitamin D supplement, have a professional occupation, had recently been on a sunny holiday abroad, and have a BMI less than 25 kg/m^2. Considering the mean tested baseline 25(OH)D concentration in our study population of asthma patients was only 45.7 nmol/L, the potential impact of just 5 environmental factors could be enough to sway levels from profound deficiency to complete repletion, or vice versa. Future studies to replicate our findings could lead to the development of a cheap screening tool for vitamin D deficiency in asthma, COPD, or older adult patient groups, which could guide general practitioners in prescribing supplements of appropriate doses in a personalised fashion, and reduce the significant cost of blanket testing vitamin D levels.

In contrast to the range of environmental factors which predict vitamin D status in our study, genetic factors show no strong association despite having been reported to do so in healthy populations; reaching GWAS level significance in some SNP (reviewed in (335)). The most obvious reason for my lack of significant findings is insufficient power to detect a signal due to our relatively small clinical trial cohorts which were powered to detect an effect of intervention on the study population. However, other studies have identified associations between vitamin D pathway SNP and 25(OH)D concentrations in similarly sized cohorts (130, 169, 378, 379), though they did only investigate several SNP and therefore any correction for multiple comparison testing they may have carried out would have had less of an impact than it did for my panel of SNP.

My analysis did not find the associations between vitamin D status and clinical features of asthma phenotype that have been shown to operate in children with asthma (245, 291-294). The cohort comprised adults with relatively well controlled asthma compared to these participants, which may explain our conflicting outcomes. Or it may be that asthma phenotype can be modified by vitamin D

supplementation in children but not in adults. Previous trials which have investigated asthma exacerbation risk with vitamin D interventions have found a reduction in children (270, 299), but not in adults (209, 300), which would support the theory. My analysis did however highlight an association between vitamin D deficiency and increased COPD severity, determined by decreased spirometric volumes: % predicted FEV_1 ($ppFEV_1$) and % predicted FVC (ppFVC) (Tables 5.4 and 5.5). These findings have been reported previously (323-328), though questions of reverse causality and/or residual confounding have been suggested. My analysis controlled for a range of factors which may potentially confound the association between vitamin D status and $ppFEV_1$/ppFVC, such as age, sex, BMI, SEP, and smoking status, which reduces the likelihood of this finding being due to residual confounding. That my analysis did not find a signal between genetic variants previously shown to associate with vitamin D status, or a signal to suggest the association between vitamin D status and $ppFEV_1$/ppFVC are modified by genetic variants (Tables 5.12 and 5.13) would suggest that vitamin D deficiency does not cause reduced $ppFEV_1$/ppFVC, however due the limitations of lacking power and/or the stringent statistical correction discussed above, I cannot exclude the possibility that genetic variants do predict vitamin D status in COPD patients and I simply failed to detect this signal. Further studies should be conducted to investigate the association between vitamin D deficiency and reduced spirometric volume in COPD patients, in a study population powered to detect the influence of genetic variation in vitamin D status and give conclusive results on the direction of effect. If we can establish that this it is forward causation, recommendations to use vitamin D supplementation to improve COPD severity can be made.

The effect of genetic variation on efficacy of vitamin D supplementation.

The major finding I present in this thesis is evidence of a haplotype of 5 *VDR* SNP which modify the effects of vitamin D supplementation on prevention of URI in COPD patients. Presence of the AACCA haplotype (Figure 9.4) independently associates with a 61% reduction in URI risk in intervention arm participants of the ViDiCO clinical trial, independent of their baseline vitamin D status (P value for

interaction = 0.001) (210). This finding was not replicated in the asthma and older adult trials, however. Given the level of significance of the finding in COPD patients, and that the 5 individual genetic variants, in high LD with each other, independently modified the effect of vitamin D supplementation after correction for multiple comparison testing, the likelihood of this being a chance finding is low. So why would this haplotype of SNP affect response to vitamin D supplementation in COPD patients, but not in asthma patients or older adults? Unlike results from COPD clinical trials (210, 272), trials in adults with asthma and older adults have not offered evidence that vitamin D supplementation is an effective intervention in prevention of ARI and/or exacerbations: two adult asthma trials report null results for protection against asthma treatment failure rates, risk of first URI, and risk of severe exacerbation (209, 300), whilst three older adult trials report null results for outcomes of self-reported ARI or antibiotic use, risk of hospital readmission due to LRI, and risk of first ARI (273) (267) (344). These trials do have limitations which might be responsible for their lack of positive findings (discussed in Chapters 3, 7, and 8) thus null results from further trials which don't suffer from the same limitations will be required to show these patient groups are non-responsive to the actions of vitamin D, and genetic variation in the vitamin D pathway is irrelevant to risk of ARI.

Whilst my investigation of variation in genes along the vitamin D pathway did highlight a haplotype in *VDR* as an effect modifier of vitamin D supplementation, it did not offer a solid explanation of the mechanism by which this haplotype affects differential risk of URI in COPD patients. Several of the AACCA haplotype SNP have been found to associate with a range of inflammatory disease outcomes (discussed in Chapter 9), and one of them (rs11568820 [cdx2]) has been reported to be functional by affecting affinity of a binding site on *VDR* for the positive transcription factor protein, cdx2, making it the most likely causal variant. RT-qPCR failed to show a significant difference in *VDR* mRNA expression level in whole blood, though I was grossly under-powered to test this in my subset of 55 participants for whom samples were available. Positive immunological analysis results were also tempered by lacking power and strict statistical correction methods, but suggested AACCA positive individuals of

the intervention arm showed decreased release of pro-inflammatory mediators, G-CSF and MIG/CXCL9, in response to stimulation with TLR ligands, which hints towards a mechanism by which vitamin D supplementation in AACCA positive individuals might result in a dampening of the hyper-inflammatory airway conditions seen in COPD patients, that lead to tissue necrosis, degranulation and lung destruction.

Further work to validate the importance of AACCA haplotype in COPD patients, and elucidate the mechanism by which possession of these variants offer a drastic reduction in risk of URI, may lead to future therapies for the disease that was responsible for 3 million deaths globally in 2012 (43).

Table 10.1. Clinical trials of vitamin D supplementation in the prevention of ARI outcomes, conducted since spring, 2012.

Author	Bergman et al. (367)	Rees et al. (373)	Marchisio et al. (368)	Tran et al. (374)	Grant et al. (371)	Goodall et al. (369)	Urashima et al. (372)	Simpson et al. (370)
Publication Year	2012	2013	2013	2013	2014	2014	2014	2015
Location	Sweden	USA	Italy	Australia	New Zealand	Canada	Japan	Australia
Journal	BMJ Open	Clin Infect Dis	Pediatr Infect Dis J	AJCN	Acta Paediatrica	BMC Infecious Diseases	Food & Fnctn	BMC Nut
Trial Registration ID	NCT01131858	NR	NR	ACTRN12609001063202	ACTRN12610000483055	NCT01158560	UMIN000002532	ACTRN12612000054819
Participants	Adults with antibody deficiency or recurrent ARI	Adults aged 45-75 with baseline 25(OH)D >12 ng/ml	Children aged 1-5 yr with recurrent acute otitis media	Healthy adults aged 60-84 at recruitment, mean age 72 years	Pregnant women and their offspring	Healthy students, age 17 and older at enrollment with at least 1 housemate	High school students aged 15-18 years	Healthy adults, mean age ~32yrs
Participants (n)	140 Intervention = 70 Control = 70	759 Intervention = 399 Control = 360	116 Intervention = 58 Control = 58	644 Intervention = 430 Control = 214	260 Intervention = 173 Control = 87	600 Intervention = 300 Control = 300	247 Intervention = 148 Control = 99	34 Intervention = 18 Control = 16
Mean / median 25(OH)D, baseline (nmol/L)	51.4	62.4	64.9	41.9	54.9 (maternal)	not measured	not measured	67.9
Dose vit D Intervention (IU)	4,000 IU	1,000 IU +/- 1200 mg calcium	1,000 IU	30,000IU, or 60,000 IU	1000/2000 IU (mothers), 400/800 IU (infants)	10,000 IU	2,000 IU	20,000 IU
Study Duration	1 year	13 months average	6 months	12 months	18 months	8 weeks	2 months	17 weeks
Frequency vit D Intervention	daily	daily	daily	monthly	daily	weekly	daily	weekly
Attained mean 25(OH)D (nmol/L)	Intervention = 133.2 Control = 66.6	Intervention = 83.1 Control = 62.6	Intervention = 91.9 Control = 46.7	Intervention = 70.9 Control = 41.9	Intervention = ~92 Control = ~77 (at 6 months)	not measured	not measured	Intervention = 76.4 Control = 60.4
Type of Randomisation	individual	individual / 2x2 Factorial	individual	individual	individual	individual / 2x2 Factorial	individual	individual
Investigated Outcome	ARI	URI	Acute otitis media (AOM)	URI	ARI	URI (primary) and lab confirmed URI (secondary)	Influenza A	ARI
Major Finding(s)	Reduced infectious score in vit D arm (202 points vs. 249 points in control arm) adjusted relative score 0.77 (95% CI 0.60 – 0.99, P=0.04)	No significant difference in winter URI rate (RR 0.93; 95% CI 0.79 – 1.09); colds rate (RR 0.93; 95% CI 0.78 – 1.10); or ILI (RR 0.95; 95% CI 0.62 – 1.46) between arms	Reduced number experiencing ≥1 AOM in intervention vs. control group (26 vs. 38; P=0.03)	No significant difference in number of prescribed antibiotics for ARI between arms (RR 0.72; 95% CI 0.48 – 1.07)	Significant reduction in proportion of ARI visits for high dose group vs. placebo (84% vs. 99%, P=0.004); non-significant reduction for low dose group vs. placebo (95% vs. 99%, P=0.17)	Significant reduction in lab confirmed URI in intervention arm (RR 0.54; 95% CI 0.34 – 0.84; P=0.007) No significant difference in reported URI between intervention and control arms or gargling and non-gargling arms	No significant difference in physician diagnosed Influenza A; significant reduction in influenza A in intervention arm after 1 month in post-hoc analysis (RR 0.17; 95% CI 0.04 – 0.77; P=0.009)	No significant difference in risk of ARI between intervention and control groups (HR 0.83; 95% CI 0.53 – 1.31); significant reduction in risk in subgroup analysis of those vit D deficient at baseline (HR 0.27; 95% CI 0.07 to 1.00; P=0.050)

Table 10.1 continued.

Author	Dubnov-Raz et al. (375)	Martineau et al. (209)	Martineau et al. (210)	Martineau et al. (313)	Castro et al. (300)	Mezawa et al.	Ginde et al.
Publication Year	2015	2015	2015	2015	2015	unpublished	unpublished
Location	NR	UK	UK	UK	USA	Japan	USA
Journal	Pediatr Exerc Sci	Thorax	Lancet Resp	Thorax	Am J Respir Crit Care Med	--	--
Trial Registration ID	NCT01158560	NCT00978315	NCT00977873	NCT01069874	NCT01248065	UMIN000004161	NCT01102374
Participants	Adolescent swimmers	Adults with asthma	Adults with COPD	Older adults and their carers	Adults with asthma and 25(OH)D < 75 nmol/L	Children with asthma aged 6-15 years	Long-term care residents (nursing home or assisted living) age >=60 years
Participants (n)	55 Intervention = 27 Control = 27	250 Intervention = 125 Control = 125	240 Intervention = 122 Control = 118	240 Intervention = 137 Control = 103	408 Intervention = 201 Control = 207	89 Intervention = 54 Control = 35	107 Intervention = 55 Control = 52
Mean / median 25(OH)D, baseline (nmol/L)	60.4	50.4	45.7	42.4	47.2	71.8	57.4
Dose vit D Intervention (IU)	2,000 IU/day	6 x 120,000 IU boluses	6 x 120,000 IU boluses	6 x 120,000 IU boluses	10,000 IU loading dose; 4,000 IU/day	800 IU	100,000 IU
Study Duration	12 weeks	1 year	1 year	1 year	6 months	2 months	12 months
Frequency vit D Intervention	daily	2 monthly	2 monthly	2 monthly	daily	daily	monthly
Attained mean 25(OH)D (nmol/L)	Intervention = 73.9 Control = 50.7	Intervention = 70.4 Control = 46.4	Intervention = 67.4 Control = 47.1	Intervention = 85.3 Control = 59.1	Intervention = 104.3 Control = 49.9 (at 28 weeks)	Intervention = 87.4 Control = 70.1	--
Type of Randomisation	individual	individual	individual	cluster	individual	individual	individual
Investigated Outcome	URI	URI and asthma exacerbation	URI and COPD exacerbation	ARI	Asthma treatment failure	Asthma exacerbation	ARI
Major Finding(s)	No significant difference between frequency, severity, or duration of URI between intervention and control groups.	No significant difference in time to first severe exacerbation (aHR 1.02; 95% CI 0.69 – 1.53; P=0.91) or first URI (aHR 0.87; 95% CI 0.64 – 1.16; P=0.34) between study arms.	No significant difference in time to first exacerbation (aHR 0.86; 95% CI 0.60 – 1.24; P=0.42) or URI (aHR 0.95; 95% CI 0.69 – 1.31; P=0.75) between arms; significant difference in time to first exacerbation in those vit D deficient at baseline (aHR 0.57; 95% CI 0.35 – 0.92, P=0.021)	No significant difference in time to first ARI between arms (aHR 1.18; 95% CI 0.80 – 1.74; P=0.42); significantly greater risk of URI (aHR 1.48; 95% CI 1.02 – 2.16; P=0.039) and duration of URI symptoms (7 vs 5 days; P=0.005) for intervention arm vs. placebo.	No significant difference in asthma treatment failure rate between study arms (aHR 0.90; 95% CI 0.60 – 1.30)	--	--

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Appendix: Publications arising from this thesis.

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Review

Vitamin D in the prevention of acute respiratory infection: Systematic review of clinical studies

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ABSTRACT

Vitamin D metabolites enhance immunity to a wide range of respiratory pathogens in vitro. Numerous observational studies have investigated whether vitamin D deficiency is a risk factor for acute respiratory infection, and a number of clinical trials of vitamin D supplementation for the prevention of acute respiratory infection have recently been conducted. Syntheses of this literature are lacking. We therefore conducted a systematic review of clinical studies investigating the association between vitamin D deficiency and susceptibility to acute respiratory infection in humans. A total of 39 studies (4 cross-sectional studies, 8 case-control studies, 13 cohort studies and 14 clinical trials) satisfying review eligibility criteria were identified. Observational studies predominantly reported statistically significant associations between low vitamin D status and increased risk of both upper and lower respiratory tract infections. Results from randomised controlled trials were conflicting however, reflecting heterogeneity in dosing regimens and baseline vitamin D status in study populations. Further trials of vitamin D supplementation for the prevention of acute respiratory infection should be conducted in populations with a high prevalence of vitamin D deficiency at baseline, using doses sufficient to induce sustained elevation of serum 25-hydroxyvitamin D concentrations, and powered to detect clinically important sub-group effects.

This article is part of a Special Issue entitled 'Vitamin D Workshop'.

Contents

Abbreviations: 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; ARI, acute respiratory infection; CI, confidence interval; COPD, chronic obstructive pulmonary disease; LRTI, lower respiratory tract infection; OR, odds ratio; RSV, respiratory syncytial virus; URTI, upper respiratory tract infection; VDR, vitamin D receptor.

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1. Introduction

Acute respiratory infections (ARI) comprise infections of the nasal cavity, paranasal sinuses, larynx, pharynx, epiglottis, trachea, bronchioles or lungs with duration ≤30 days. They are classically categorised by site, with infections above the vocal cords classified as upper respiratory tract infections (URTI), and those below the vocal cords classified as lower respiratory tract infections (LRTI). Examples of URTI include nasopharyngitis, sinusitis, pharyngitis, tonsillitis, laryngitis and tracheitis, whilst bacterial and viral pneumonia, acute bronchitis and bronchiolitis are categorised as LRTI; influenza may present as both an upper and lower respiratory tract infection [1]. ARI are the third most common cause of mortality globally, responsible for an estimated 4.26 million deaths in 2004; 98% of these deaths were due to LRTI [2]. Vaccination is the mainstay of prevention, but effective vaccines are unavailable for several important respiratory pathogens; moreover, availability of existing vaccines in low income settings that bear the brunt of morbidity and mortality is limited. Alternative low-cost interventions with potential to protect against multiple respiratory pathogens are urgently needed.

A growing body of evidence suggests that vitamin D supplementation may represent one such intervention [3]. Humans synthesise vitamin D_3 (cholecalciferol) in the skin following exposure to ultra-violet B radiation; a relatively small amount of the human vitamin D requirement is met by dietary intake (primarily from oily fish). Vitamin D is 25-hydroxylated in the liver to produce 25-hydroxyvitamin D (25[OH]D), the major circulating vitamin D metabolite and widely accepted measure of vitamin D status: a serum 25(OH)D concentration <20 ng/ml constitutes deficiency [4]. 25(OH)D undergoes 1-alpha-hydroxylation to produce 1,25-dihydroxyvitamin D ($1,25[OH]_2D$), the secosteroid hormone that ligates vitamin D receptor (VDR) with high affinity to mediate the biological actions of vitamin D. The 1-alpha hydroxylase enzyme, CYP27B1, that catalyses this activation step is expressed in the kidney and in a variety of extra-renal tissues, including leucocytes and pulmonary epithelium, and its expression is induced by both viral and bacterial ligands [5,6]. Thus, $1,25[OH]_2D$ can be synthesised in the lung in response to pulmonary infection when 25(OH)D substrate is available (i.e. in vitamin D replete individuals). VDR is widely expressed in cells of the immune system and the respiratory tract, and $1,25[OH]_2D$ ligates it to induce broad-spectrum antimicrobial responses that are effective against both viral and bacterial respiratory pathogens [7,8]. Elucidation of these immunomodulatory actions of vitamin D in vitro has prompted the conduct of numerous observational and interventional studies investigating the influence of in vivo vitamin D status on susceptibility to ARI, but syntheses of this literature are lacking. We therefore conducted a systematic review of pertinent clinical studies.

2. Methods

2.1. Search method

The PubMed database was searched on 17th October 2012 using the terms 'vitamin D' with 'respiratory infection', 'COPD exacerbation', or 'asthma exacerbation'. No restrictions were placed on language of publication or on the age, sex, ethnic origin, baseline vitamin D status or presence or absence of comorbidity in populations studied.

Studies were classified into one of three categories: potentially eligible primary studies, relevant review articles and ineligible primary studies. Full text of potentially eligible primary studies was reviewed to confirm eligibility according to criteria presented below. Relevant review articles were retrieved and screened for additional primary studies. All articles were assessed for eligibility by one author (DAJ) then re-assessed by a second (ARM).

2.2. Inclusion/exclusion criteria

Studies were screened by title and abstract to evaluate whether they met the following eligibility criteria:

2.2.1. Inclusion criteria

- Cross-sectional studies, case-control studies, cohort studies or clinical trials conducted in human subjects, and investigating the relationship between serum concentration of vitamin D metabolites or clinical manifestations of vitamin D deficiency, or effect of dietary intake or administration of vitamin D or its analogues, on risk of acute respiratory infection or acute exacerbation of asthma or Chronic Obstructive Pulmonary Disease (COPD).

2.2.2. Exclusion criterion

- Studies relating exclusively to tuberculosis (these are reviewed elsewhere [9], and were beyond the remit of this review as tuberculosis is classically regarded as a chronic respiratory tract infection, with symptom duration usually exceeding 30 days).

3. Results

3.1. Identification and selection of studies

Fig. 1 depicts the study selection process. Our initial search identified 406 publications, of which 31 were initially identified as fulfilling eligibility criteria listed above. A further 8 eligible primary manuscripts were identified from the 39 relevant reviews identified in the initial search, bringing the total number of eligible studies for inclusion in this systematic review to 39.

3.2. Study characteristics

Of the 39 studies reviewed, 25 are observational studies (4 cross-sectional, 8 case-control and 13 cohort) and 14 are intervention studies (all randomised controlled trials). The selected studies report data from a total of 47,360 participants whose age range from newly born to >80 years old. Studies were conducted in USA (7 studies), UK (4), Canada (3), Japan (3), Afghanistan (2), Finland (2), India (2), New Zealand (2), Bangladesh, Belgium, Ethiopia, Germany, Jordan, Mongolia, the Netherlands, Norway, Poland, Puerto Rico, Romania, Saudi Arabia, Spain and Turkey (1 study each). Thirty-one studies report serum 25(OH)D concentrations, one study reports serum $1,25(OH)_2D$ concentrations, and seven studies do not report concentrations of either metabolite.

3.3. Study findings

Table 1 presents results of the four cross-sectional studies reviewed: all report consistent associations between low serum 25(OH)D concentrations and increased risk of ARI [10–13]. Results of the eight case-control studies reviewed are presented in Table 2: five of these studies report associations between susceptibility to ARI and vitamin D deficiency, as evidenced by the presence of rickets [14,15] or by low serum 25(OH)D concentrations [16–18]; one reports an association between low vitamin D intake and increased risk of LRTI [19], and two report no association between serum 25(OH)D concentration and susceptibility to LRTI [20,21]. Table 3 presents results of the thirteen cohort studies reviewed: seven report associations between low serum 25(OH)D concentrations and susceptibility to ARI [22–28], two suggest that serum $1,25(OH)_2D$ concentrations may be protective (as evidenced by higher serum $1,25(OH)_2D$ concentrations [29] or by administration

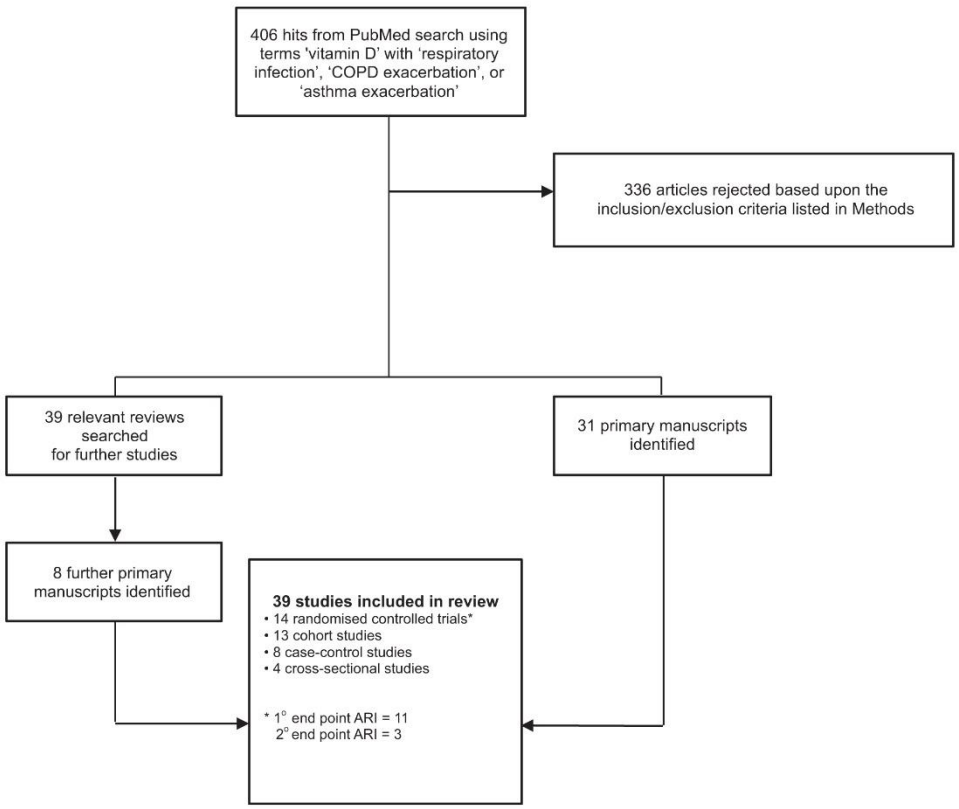

Fig. 1. Study selection.

Table 1
Cross-sectional studies investigating association between vitamin D status and susceptibility to acute respiratory infection.

First author, year, setting	Participants	Serum 25(OH)D concentration	Main findings
Ginde 2009. USA [10]	18,883 survey participants, median age 38 years	Median 29.0 ng/ml 3.6% <10 ng/ml 65.1% 10–29 ng/ml 31.3% ≥30 ng/ml	• Serum 25(OH)D concentration was inversely associated with risk of self-reported recent URTI symptoms (OR 1.36, 95% CI 1.01–1.84, for participants with 25(OH)D <10 ng/ml vs. those with 25(OH)D ≥30 ng/ml) • Inverse associations between serum 25(OH)D concentration and risk of URTI were stronger in individuals with asthma (OR 5.67) and COPD (OR 2.26)
Jarrti, 2010. USA [11]	284 hospitalised wheezing children, median age 1.6 years	Mean 27.2 ng/ml	• Serum 25(OH)D concentration was inversely associated with risk of RSV infection (OR per 4 ng/ml increase, 0.91; 95% CI 0.83–0.99), rhinovirus (OR per 4 ng/ml increase, 0.92; 95% CI 0.85–0.99) and multiple viral cause (OR per 4 ng/ml increase, 0.91; 95% CI 0.84–0.99)
Berry, 2011. UK [12]	6789 survey participants aged 45 years	Mean 29.0 ng/ml 7.7% <10 ng/ml 69.8% 10–29 ng/ml 22.6% ≥30 ng/ml	• Serum 25(OH)D was inversely associated with risk of acute respiratory infection (after adjustment for adiposity, lifestyle and socio-economic factors, each 4 ng/ml increase in 25(OH)D associated with a 7% lower risk of self-reported ARI; 95% CI, 3–11%; P for trend <0.001)
Brehm, 2012. Puerto Rico [13]	287 children with asthma aged 6–14 years	Mean 32.0 ng/ml 44% <30 ng/ml 56% ≥30 ng/ml	• Serum 25(OH)D concentration <30 ng/ml associated with higher odds of ≥1 severe asthma exacerbation in the prior year in multivariate analysis (OR 2.6, 95% CI 1.5–4.9, $P = 0.001$)

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; URTI: Upper Respiratory Tract Infection; OR; odds ratio; CI: confidence interval; RSV: respiratory syncytial virus; ARI: acute respiratory infection. 25(OH)D concentrations converted from nmol/L to ng/ml by dividing by 2.496.

Table 2
Case-control studies investigating association between vitamin D status and susceptibility to acute respiratory infection.

First author, year, setting	Participants	Mean serum 25(OH)D concentration		Main findings
Muhe, 1997. Ethiopia [14]	1000 children, mean age 13 months: 500 rickets cases vs. 500 healthy controls	Not measured		• Diagnosis of rickets associated with susceptibility to pneumonia (adjusted OR 13·37; 95% CI, 8·08–24·22; $P<0·001$)
Najada 2004. Jordan [15]	443 acutely hospitalised children aged 3–24 months: 47 cases vs. 396 controls without rickets	Not measured		• Diagnosis of rickets associated with risk of LRTI (85% of children with rickets had LRTI vs. 30% of children without rickets, $P<0.01$)
Wayse, 2004. India [16]	150 children, mean age 23.9 months: 80 cases with LRTI vs. 70 healthy controls	Cases, 9.1 ng/ml	Controls, 15.4 ng/ml	• Serum 25(OH)D concentration was significantly lower in cases vs. controls ($P<0.001$) • Serum 25(OH)D concentration >9.0 ng/ml associated with decreased risk of LRTI (adjusted OR 0.09; 95% CI, 0.03 to 0.24; $P<0.001$)
Karatekin, 2009. Turkey [17]	40 neonates: 25 cases admitted to neonatal intensive care with LRTI vs. 15 healthy controls	Cases, 9.1 ng/ml	Controls, 16.3 ng/ml	• Mean serum 25(OH)D concentration was significantly lower in cases with LRTI vs. healthy controls ($P=0.01$) • Serum 25(OH)D < 10 ng/ml associated with increased risk of LRTI (OR 4.25; 95% CI, 1.06–17.07; $P=0.04$)
Roth, 2009. Canada [20]	129 children, mean age 13 months: 64 cases hospitalised with uncomplicated LRTI vs. 65 healthy controls	Cases, 30.9 ng/ml	Controls, 30.8 ng/ml	• No significant difference in mean serum 25(OH)D concentration between LRTI cases vs. controls ($P=0.96$) • Inadequate vitamin D status was not associated with the risk of LRTI at either 16 ng/ml or 32 ng/ml 25(OH)D thresholds ($P \geq 0.37$)
McNally, 2009. Canada [21]	197 children, mean age 14 months: 105 cases hospitalised with LRTI vs. 92 controls attending hospital with other diagnosis	Cases, 32.5 ng/ml	Controls, 33.3 ng/ml	• No significant difference in mean serum 25(OH)D concentration between cases vs. controls ($P=0.71$) • Among cases, serum 25(OH)D <20 ng/ml associated with increased risk of admission to the intensive care unit (adjusted OR 8.23, 95% CI, 1.4–48.0, $P=0.02$)
Roth, 2010. Bangladesh [18]	50 children aged 1–18 months: 25 cases hospitalised with LRTI vs. 25 healthy controls	Cases, 11.7 ng/ml	Controls, 15.7 ng/ml	• Mean serum 25(OH)D concentration was significantly lower in LRTI cases vs. healthy controls ($P=0.015$) • Adjusted odds for LRTI was reduced 4.3-fold for every 4 ng/ml increase in serum 25(OH)D concentration (adjusted OR 0.23; 95% CI, 0.06–0.81; $P=0.02$)
Leis, 2012. Canada [19]	197 children aged <5 years: 105 cases hospitalised with LRTI vs. 92 controls attending hospital with other diagnosis	Not presented	Not presented	• Vitamin D intake <80 IU/kg/day associated with increased risk of LRTI (adjusted OR 4.9, 95% CI 1.5–16.4, $P=0.01$)

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; OR: odds ratio; CI: confidence interval; LRTI: Lower Respiratory Tract Infection. 25(OH)D concentrations converted from nmol/L to ng/ml by dividing by 2.496.

of 1-alpha-hydroxylated vitamin D metabolites [30]), three studies are null [31–33], and one reports a positive association between high maternal serum 25(OH)D concentration in late pregnancy and increased risk of LRTI in offspring during infancy [34]. Results of the fourteen clinical trials reviewed are presented in Table 4: ARI was primary outcome in eleven of these studies, and a secondary outcome in three. Seven trials report that vitamin D supplementation protected against ARI–six in the study population as a whole [35–40], and one in a sub-group with profound vitamin D deficiency [41]. Six trials report null effects for all respiratory outcomes investigated [42–47], and one reports a null effect of vitamin D supplementation on primary outcome (pneumonia incidence), with a negative effect on one secondary outcome (vitamin D increased incidence of repeat episodes of radiographically confirmed pneumonia) [48].

4. Discussion

This systematic review has identified broadly consistent evidence of an association between inadequate vitamin D status and susceptibility to ARI in observational studies conducted in large numbers of participants of all ages in diverse geographical settings and with a wide distribution of serum 25(OH)D concentrations. Evidence from intervention studies is more conflicting, with seven of the trials reviewed reporting protective effects of vitamin D supplementation, six reporting null effects, and one reporting an adverse effect of vitamin D supplementation on risk of pneumonia recurrence. Two interpretations of this 'disconnect' in the evidence from observational vs. intervention studies may be made. Associations seen in observational studies may be attributed to confounding, and inconsistent results from trials may be interpreted as providing insufficient evidence of effectiveness of vitamin D supplementation in preventing ARI. Alternatively it may be argued that associations reported in observational studies are indeed causal, and that negative results in some trials have arisen as a result of high baseline vitamin D status in trial participants and/or ineffective vitamin D supplementation regimens employed in these studies. We subscribe to the latter interpretation, and a consideration of the strengths and limitations of the studies that we have reviewed therefore follows.

Table 3
Cohort studies investigating association between vitamin D status and susceptibility to acute respiratory infection.

First author, year, setting	Design	Participants	Duration of follow-up	Serum 25(OH)D concentration	Main findings
Laaksi, 2007. Finland [22]	Prospective	756 male military recruits, aged 18–29 years	6 months	Mean, 32.1 ng/ml 3.6% <16 ng/ml	• Subjects with 25(OH)D <16 ng/ml had more days of absence from duty due to respiratory infection than those with 25(OH)D ≥16 ng/ml (incidence rate ratio 1.63; 95% CI, 1.15–2.24, $P=0.004$)
Gale, 2008. UK [34]	Prospective (birth cohort)	466 mothers (mean age not reported) and 466 infants	9 months	Mean maternal 25(OH)D at late pregnancy, 20 ng/ml 21.2% <11 ng/ml 28.3% 11–20 ng/ml 50.4% >20 ng/ml	• Maternal serum 25(OH)D concentration in the top quartile (>30 ng/ml) vs. bottom quartile (<12 ng/ml) associated with increased risk of pneumonia or bronchiolitis in offspring (OR 4.80, 95% CI 1.01–22.72) • 'Overall' maternal serum 25(OH)D concentration did not associate with risk of ARI in offspring (OR not presented)
Asamura, 2010. Japan [29]	Retrospective	32 nursing home residents, mean age 80.9 years	2 years	Not presented	• Serum $1,25(OH)_2D$ concentration inversely associated with risk of febrile respiratory illness (64% in those with $1,25(OH)_2D$ <42 pg/ml vs. 22% in those with $1,25(OH)_2D$ ≥42 pg/ml, $P=0.03$) • Serum 1,25(OH)2D concentration did not significantly associate with risk of pneumonia (21% in those with $1,25(OH)_2D$ <42 pg/ml vs. 6% in those with $1,25(OH)_2D$ ≥42 pg/ml, $P=0.30$)
Sabetta, 2010. USA [23]	Prospective	198 healthy adults aged 20–88 years	4 months	Mean, 28.4 ng/ml 90.9% <38 ng/ml 9.1% ≥38 ng/ml	• Serum 25(OH)D concentration inversely associated with risk of viral ARTI (45% in those with 25(OH)D <38 ng/ml vs. 17% in those with serum 25(OH)D ≥38 ng/ml, $P=0.01$) • Serum 25(OH)D concentration did not associate with median illness duration (6 days in those with serum 25(OH)D <38 ng/ml vs. 6 days in those with serum 25(OH)D ≥38 ng/ml (P value not presented)
Brehm, 2010. USA [28]	Prospective	1024 children with mild-moderate asthma, median age 8.9 years	4 years	35% ≤ 30 ng/ml 65% >30 ng/ml	• Serum 25(OH)D concentration ≤ 30 ng/ml associated with higher odds of any hospitalization or emergency department visit (OR 1.5; 95% CI, 1.1–1.9; $P=0.01$)
Camargo, 2011. New Zealand [24]	Prospective (birth cohort)	922 neonates	5 years	Cord blood concentrations: Median, 17.6 ng/ml 19.5% <10 ng/ml 53.3% 10–29 ng/ml 27.2% ≥30 ng/ml	• Serum 25(OH)D concentration inversely associated with risk of ARI by 3 months of age (OR 1.0 for ≥30 ng/ml, 1.39 for 10–30 ng/ml, 2.16 for 25(OH)D <10 ng/ml, P for trend 0.004) • Cord-blood 25(OH)D levels inversely associated with risk of wheezing by 15 months, 3 years and 5 years of age ($P<0.05$)
Tsujimoto, 2011. Japan [30]	Retrospective	508 haemodialysis patients, mean age 59.6 years. 212 took alfacalcidol or calcitriol, 296 did not	5 years	Not presented	• Administration of alfacalcidol or calcitriol was associated with reduced risk of hospitalisation with LRTI (adjusted hazard ratio: 0.47; 95% CI 0.25–0.90; $P=0.02$)
Belderbos, 2011. Netherlands [25]	Prospective (birth cohort)	156 neonates	1 year	Mean, 32.9 ng/ml 23.1% <20 ng/ml 30.8% 20–29 ng/ml 46.1% ≥30 ng/ml	• Serum 25(OH)D concentration at birth inversely associated with risk of RSV LRTI over 1st year of life (adjusted relative risk 6.2, 95% CI 1.6 to 24.9, P = 0.01 for neonates with 25(OH)D <20 ng/ml vs. 25(OH)D≥ 30 ng/ml)
Porojnicu 2012. Romania [31]	Prospective	105 healthy hospital employees, mean age 35.3 years	2 months	35% <12 ng/ml 45% 12–19 ng/ml 17% 20–32 ng/ml 3% ≥32 ng/ml	• Serum 25(OH)D concentration did not significantly associate with self-reported cases of ARI (Spearman coefficient for correlation between 25(OH)D concentration and number of infectious episodes, −0.12; $P=0.20$)
Morales, 2012. Spain [26]	Prospective (birth cohort)	1724 infants	6 years	Median maternal 25(OH)D at 12 weeks' gestation, 29.5 ng/ml	• Maternal serum 25(OH)D concentration at 12 weeks' gestation inversely associated with risk of LRTI in offspring at 1 year (OR 0.67, 95% CI 0.50–0.90, $P=0.02$ for highest vs. lowest quartile of maternal 25(OH)D)
Kunisaki 2012. USA [33]	Prospective	973 COPD patients	1 year	Mean, 25.7 ng/ml 8.4% <10 ng/ml 23.6% 10–19 ng/ml 33.1% 20–29 ng/ml 34.9% ≥ 30 ng/ml	• Serum 25(OH)D concentration did not associate with time to first acute exacerbation of COPD (hazard ratio for a 10 ng/ml increment in 25(OH)D, 1.04, 95% CI 0.97–1.12)
Mohamed 2012. Saudi Arabia [27]	Prospective	206 infants	2 years	Mean cord blood concentration, 24.1 ng/ml 12% <12 ng/ml 18% 12–19 ng/ml 26% 20–29 ng/ml 44% ≥30 ng/ml	• Mean cord blood 25(OH)D concentration was lower among infants who developed LRTI in the first 2 years of life vs. those who did not (13.6 ng/ml vs. 28.6 ng/ml, $P<0.0001$) • In multivariate analysis, low cord blood 25(OH)D concentration independently associated with subsequent risk of ALRI (OR 1.08; 95% CI 1.05–1.10; $P<0.001$)
Quint, 2012. UK [32]	Prospective	97 COPD patients	12 months	Median (IQR), Summer: 16.5 ng/ml (10.7–26.0 ng/ml) Winter: 11.1 ng/ml (7.8–17.8 ng/ml)	• Serum 25(OH)D concentration did not associate with risk of COPD exacerbation during summer or winter (median serum 25[OH]D concentration for frequent vs. infrequent exacerbators in summer: 17.7 ng/ml vs. 15.8 ng/ml; winter: 10.0 ng/ml vs. 10.9 ng/ml, respectively; $P\geq 0.21$)

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; $1,25(OH)_2D$: 1,25-dihydroxyvitamin D; OR: odds ratio; CI: confidence interval; RTI: Respiratory Tract Infection; LRTI: Lower Respiratory Tract Infection. COPD: Chronic Obstructive Pulmonary Disease; 25(OH)D concentrations converted from nmol/L to ng/ml by dividing by 2.496.

Table 4
Clinical trials investigating effects of vitamin D supplementation on incidence of acute respiratory infection.

First author, year, setting	Participants	Duration of follow-up	Dose of vitamin D_3, intervention arm	Mean serum 25(OH)D concentration, intervention arm	Mean serum 25(OH)D concentration, placebo arm	Main findings
Aloia 2007. USA [35]	208 healthy post-menopausal African–American women aged 50–75 years; 104 allocated to intervention, 104 to placebo	3 years	800 IU/day for 2 years, then 2000 IU/day for 1 year	Intervention: Baseline, 19.3 ng/ml 3 months, 28.4 ng/ml 27 months, 34.8 ng/ml	Placebo: Baseline, 17.2 ng/ml Follow-up: "no significant change throughout the study"	• Allocation to intervention arm decreased rate of self-reported URTI symptoms (8% intervention arm vs. 25% placebo arm, $P < 0.002$)
Avenell, 2007. UK [42]	3,444 adults aged ≥70 years; 1740 allocated to receive vitamin $D_3 \pm$ calcium, 1704 receiving placebo ± calcium	2 years	800 IU/day, 1000 mg calcium/day, 800 IU/day + 1000 mg calcium/day, or placebo	Intervention: Baseline, 15.2 ng/ml (subset of $n = 60$ intervention members) 12 months, 24.8 ng/ml (same subset)	Not Presented	• Allocation to intervention arm did not affect incidence of self-reported infections (OR 0.90; 95% CI, 0.76–1.07; $P = 0.23$) or antibiotic use (OR 0.84; 95% CI, 0.64–1.09; $P = 0.18$)
Li-Ng, 2009. USA [43]	162 healthy adults, mean age 59 years; 84 allocated to intervention, 78 allocated to placebo	3 months	2.000 IU/day	Intervention: Baseline, 25.8 ng/ml 3 months, 35.5 ng/ml	Placebo: Baseline, 25.2 ng/ml 3 months, 24.4 ng/ml	• Allocation to intervention arm did not affect rate of self-reported URTI (12% intervention vs. 14% placebo arm, $P = 0.56$), URTI duration (mean duration 5.4 days in intervention arm vs. 5.3 days in placebo arm, $P = 0.86$) or URTI severity (mean severity score 2.6 in intervention arm vs. 2.8 in placebo arm, $P = 0.40$)
Bischoff-Ferrari, 2010. Germany [36]	173 patients with recent hip fracture, mean age 84 years; 86 allocated to higher dose vitamin D_3, 87 allocated to lower dose vitamin D_3	1 year	2000 IU/day vs. 800 IU/day	2000 IU/day group: Baseline, 13.1 ng/ml 6 months, 45.4 ng/ml 12 months, 44.7 ng/ml	800 IU/day group: Baseline, 12.1 ng/ml 6 months, 37.7 ng/ml 12 months, 35.4 ng/ml	• Allocation to higher dose vitamin D_3 decreased the risk of hospital readmission due to infection at any site (1% in higher dose group vs. 11% in lower dose group; adjusted relative rate difference −90, 95% CI −99 to −13) • Allocation to higher dose vitamin D did not influence risk of hospital readmission due to LRTI (0% in higher dose group vs. 2% in lower dose group, $P = 0.16$)
Urashima 2010. Japan [37]	334 school children, aged 6–15 years; 167 allocated to intervention, 167 allocated to placebo	4 months	1200 IU/day	Not presented	Not presented	• Allocation to intervention arm significantly reduced risk of influenza A infection (10.8% intervention arm vs. 18.6% placebo arm; RR: 0.58; 95% CI, 0.34–0.99; $P = 0.04$)
Laaksi, 2010. Finland [44]	164 military conscripts, aged 18–28 years; 80 allocated to intervention, 84 allocated to placebo	6 months	400 IU/day	Intervention: Baseline, 31.5 ng/ml 6 months, 28.7 ng/ml	Placebo: Baseline, 29.8 ng/ml 6 months, 20.6 ng/ml	• Allocation to intervention arm did not affect the mean number of days absent from duty due to ARTI (2.2 in intervention arm vs. 3.0 in placebo arm, $P = 0.10$), or the rate of common cold symptoms (56% intervention arm vs. 52% placebo arm) • Proportion of participants 'remaining healthy' was higher in intervention vs. placebo arm (51% vs. 36% respectively, $P = 0.05$)
Manaseki-Holland, 2010. Afghanistan [38]	453 children diagnosed with pneumonia, aged 1–36 months; 224 allocated to intervention, 229 allocated to placebo	3 months	Single bolus dose of 100,000 IU	Not presented	Not presented	• Allocation to intervention arm reduced the risk of repeat LRTI episode within 90 days of randomisation (RR 0.78; 95% CI, 0.64–0.94; $P = 0.01$) • Allocation to intervention arm did not affect the mean number of days to recovery (4.7 days in intervention arm vs. 5.0 days in placebo arm; $P = 0.17$)

Table 4 (*Continued*)

First author, year, setting	Participants	Duration of follow-up	Dose of vitamin D_3, intervention arm	Mean serum 25(OH)D concentration, intervention arm	Mean serum 25(OH)D concentration, placebo arm	Main findings
Kumar, 2011. India [45]	2079 low birthweight infants born at >37 weeks' gestation; 1039 allocated to intervention, 1040 allocated to placebo	6 months	1400 IU/week	Intervention: Baseline, not presented 6 months, 22.0 ng/ml	Placebo: Baseline, not presented 6 months, 14.4 ng/ml	• Allocation to intervention arm did not affect incidence of pneumonia or incidence of all-cause hospital admission or death (adjusted rate ratio 0.98, 95% CI 0.70–1.38, $P = 0.92$)
Majak, 2011. Poland [39]	48 children with budesonide-treated asthma aged 5–18 years; 24 allocated to intervention, 24 allocated to placebo	6 months	500 IU/day	Intervention: Baseline, 36.1 ng/ml 6 months, 37.6 ng/ml	Placebo: Baseline, 35.1 ng/ml 6 months, 31.9 ng/ml	• Allocation to the intervention arm significantly decreased the risk of asthma exacerbation (17% intervention arm vs. 46% placebo arm, $P = 0.03$)
Lehouck, 2012. Belgium [41]	182 patients with moderate to severe COPD, mean age 68 years; 91 allocated to intervention, 91 allocated to placebo	1 year	Monthly bolus dose of 100,000 IU	Intervention: Baseline, 20.1 ng/ml 12 months, 52.0 ng/ml	Placebo: Baseline, 19.8 ng/ml 12 months, 20.4 ng/ml	• Allocation to intervention arm did not influence time to first exacerbation (HR 1.10; 95% CI, 0.82–1.56, $P = 0.41$) • Subgroup analysis of participants with baseline 25(OH)D <10 ng/ml ($n = 30$) showed reduced annual exacerbation rate in the intervention arm (rate ratio 0.57, 95% CI 0.33–0.98, $P = 0.04$)
Manaseki-Holland, 2012. Afghanistan [48]	3046 children aged 1–11 months; 1524 allocated to intervention, 1522 allocated to placebo	18 months	3-monthly bolus dose of 100,000 IU	Intervention: Baseline, not presented 1 week, 51.9 ng/ml 1.5 months, 30.6 ng/ml 3 months, 22.2 ng/ml 6.5 months, 42.0 ng/ml 22 months, 20.8 ng/ml	Placebo: Baseline, not presented 1 week, 17.2 ng/ml 1.5 months, 13.2 ng/ml 3 months, 15.9 ng/ml 6.5 months, 21.2 ng/ml 22 months, 20.1 ng/ml	• Allocation to intervention arm did not affect the incidence of first or only pneumonia (incidence rate ratio 1.06, 95% CI 0.89–1.27, $P = 0.48$), but did increase incidence of repeat episodes of radiographically–confirmed pneumonia (incidence rate ratio 1.69, 95% CI 1.28–2.21, $P < 0.0001$)
Jorde, 2012. Norway [46]	569 participants of 10 different clinical trials, median age 63 years; 289 allocated to intervention, 280 allocated to placebo	6 months	1111–6800 IU/day	Not presented	Not presented	• Allocation to intervention arms did not influence risk of influenza-like illness (13% intervention arms vs. 15% placebo arms, $P = 0.52$)
Camargo, 2012. Mongolia [40]	247 schoolchildren, mean age 10 years; 141 allocated to intervention, 103 to placebo	7 weeks	300 IU/day	Intervention: (median) Baseline, 7 ng/ml 7 weeks, 18.9 ng/ml	Placebo: (median) Baseline, 6.8 ng/ml 7 weeks, 7.2 ng/ml	• Allocation to intervention arm vs. placebo arm halved the rate of maternally reported ARI (adjusted RR: 0.50, 95% CI 0.28–0.88)
Murdoch, 2012. New Zealand [47]	322 healthy adults, mean age 47 years; 161 allocated to intervention, 161 allocated to placebo	18 months	Bolus dose of 200,000 IU in months 1 and 2, bolus dose of 100,000 IU/month thereafter	Intervention: Baseline, 29 ng/ml 18 months, ~50 ng/ml	Placebo: Baseline, 28 ng/ml 18 months, ~22 ng/ml	• Allocation to intervention arm had no effect on the number of URTI (RR: 0.97; 95% CI, 0.85–1.11) or duration of URTI symptoms (RR:0.96; 95% CI, 0.81–1.30)

Abbreviations: URTI: Upper Respiratory Tract Infection; LRTI: Lower Respiratory Tract Infection; ARI: Acute Respiratory Infection; HR: Hazard Ratio; RR: Risk Ratio; COPD: Chronic Obstructive Pulmonary Disease. 25(OH)D concentrations converted from nmol/L to ng/ml by dividing by 2.496.

4.1. Observational studies: strengths and limitations

Many of the observational studies reviewed here were of good quality, and potential confounders of the relationship between vitamin D deficiency and susceptibility to ARI such as age, sex, season, socioeconomic position and smoking were controlled for in the majority. Cohort studies were well represented, allowing confirmation that vitamin D deficiency precedes the onset of ARI, and does not arise as a consequence of infection, as seen with other micronutrients [49]. Most studies reported participants' serum 25(OH)D concentrations, the gold standard measurement of vitamin D status; however, two studies classified participants as vitamin D deficient on the basis of a clinical diagnosis of rickets [14,15] (which may arise in children with adequate serum 25(OH)D concentrations [50]), and one reported serum concentrations of $1,25(OH)_2D$ [29] (which are not generally considered to reflect vitamin D status [51]). Although some studies complemented symptom-based case definitions with physician, radiological, serological and/or molecular diagnosis, many utilised symptom-based definitions, which are more subjective and which cannot inform the question of whether protective effects of vitamin D are pathogen-specific, as suggested by some studies [11,37]. It should also be noted that some of the outcomes classified as ARI for the purposes of this review, such as exacerbations of asthma and COPD, do not always have an infectious aetiology.

4.2. Intervention studies: strengths and limitations

All of the clinical trials reviewed here were randomised, double-blind and placebo-controlled: this 'gold standard' study design effectively eliminates the potential for confounding and observer bias to explain any positive findings. However, some important limitations should be noted. First, some trials investigated effects of vitamin D supplementation on ARI as a secondary outcome [36], or in post hoc analyses [35,42]. Where these analyses were not prespecified in the protocol, false positive results may have arisen as a result of type 1 error, and false negative results may have arisen as a result of type 2 error or as a result of inadequate ascertainment of ARI. In the post hoc analysis conducted by Aloia et al. [35], for example, the number of URTI reported is significantly lower than would be expected for a study population followed over 3 years.

A second limitation relates to the dosing regimens used in some trials: in some cases, these were inadequate to induce prolonged, clinically significant elevations in serum 25(OH)D concentrations among participants in the intervention arm [44,45]. In others, the duration of administration and follow-up was inadequate to allow prolonged vitamin D repletion among participants in the intervention arm [43]. A further potential issue concerns the practice of administration of large intermittent bolus doses of vitamin D. This results in a steep and rapid increase in circulating 25(OH)D levels - to supra-physiological concentrations in some cases – followed by a slow decline [52]. Such peaks and troughs could have potentially deleterious effects on the immune response: concentrations of 25(OH)D >56 ng/ml have been associated with impaired immunity to infection [53], possibly reflecting the fact that vitamin D may suppress adaptive responses to infection as well as boost innate responses [54]. Moreover, chronic exposure to falling 25(OH)D concentrations has been postulated to cause an imbalance between the activity of enzymes which synthesise and catabolise $1,25(OH)_2D$ in extra-renal tissues, resulting in reduced concentrations of this active metabolite at sites of disease [55]. Either or both of these phenomena could have contributed to the excess of recurrent pneumonia observed in the intervention arm of the second trial conducted by Manaseki-Holland [48]. Administration of lower doses of vitamin D at more frequent intervals induces sustained elevation of 25(OH)D concentrations into the physiological range, and this might have more favourable effects on immune function [56]. However, if immune defects associated with vitamin D deficiency are mediated via effects on DNA methylation or histone modification, they may not be rapidly reversible by correction of deficiency.

A third limitation relates to the baseline vitamin D status of trial participants: in some cases the minority of participants were deficient [43,44,47], while in others [37,38] baseline vitamin D status was not measured, precluding identification of potentially important sub-group effects. Recently, Lehouck et al. reported that protective effects of vitamin D supplementation on COPD exacerbation were restricted to participants with baseline 25(OH)D <10 ng/ml [41], suggesting that effects of vitamin D supplementation may be dependent on baseline vitamin D status, and that the 25(OH)D threshold for protection against ARI may be low. Clinical studies conducted in patients with tuberculosis suggest that the influence of vitamin D status on antimicrobial immunity may be modified by genetic variation in the vitamin D receptor [57] and vitamin D binding protein [58]; however, none of the studies reviewed investigated this possibility. Additionally, pathogens were not characterised in many of the trials reviewed here, precluding investigation of the possibility that protective effects of vitamin D supplementation are pathogen-specific.

5. Conclusions

This systematic review has demonstrated broadly consistent associations between vitamin D deficiency and susceptibility to ARI. By contrast, results of vitamin D supplementation trials did not demonstrate consistent protective effects against ARI. Null results of clinical trials may have arisen as a result of sub-optimal vitamin D supplementation regimens and low prevalence of baseline vitamin D deficiency among participants in some trials. Further trials of vitamin D supplementation for the prevention of ARI should be conducted in populations with a high prevalence of deficiency at baseline, using doses sufficient to induce sustained elevation of serum 25(OH)D concentrations, and powered to detect clinically important sub-group effects.

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Review

Single nucleotide polymorphisms in the vitamin D pathway associating with circulating concentrations of vitamin D metabolites and non-skeletal health outcomes: Review of genetic association studies

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ABSTRACT

Polymorphisms in genes encoding proteins involved in vitamin D metabolism and transport are recognised to influence vitamin D status. Syntheses of genetic association studies linking these variants to non-skeletal health outcomes are lacking. We therefore conducted a literature review to identify reports of statistically significant associations between single nucleotide polymorphisms (SNP) in 11 vitamin D pathway genes (*DHCR7*, *CYP2R1*, *CYP3A4*, *CYP27A1*, *DBP*, *LRP2*, *CUB*, *CYP27B1*, *CYP24A1*, *VDR* and *RXRA*) and non-bone health outcomes and circulating levels of 25-hydroxyvitamin D (25[OH]D and 1,25-dihydroxyvitamin D ($1,25[OH]_2D$). A total of 120 genetic association studies reported positive associations, of which 44 investigated determinants of circulating 25(OH)D and/or $1,25(OH)_2D$ concentrations, and 76 investigated determinants of non-skeletal health outcomes. Statistically significant associations were reported for a total of 55 SNP in the 11 genes investigated. There was limited overlap between genetic determinants of vitamin D status and those associated with non-skeletal health outcomes: polymorphisms in *DBP*, *CYP2R1* and *DHCR7* were the most frequent to be reported to associate with circulating concentrations of 25(OH)D, while polymorphisms in *VDR* were most commonly reported to associate with non-skeletal health outcomes, among which infectious and autoimmune diseases were the most represented.

Contents

1. Introduction

Genetic variation in the vitamin D pathway was first reported to influence human health more than 20 years ago, when Morrison and colleagues found associations between allelic variants in the gene encoding the vitamin D receptor (VDR) and bone density [1,2].

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Since then the scope of genetic association studies in the vitamin D field has widened to investigate the effects of variation in other genes in the vitamin D pathway on both skeletal and non-skeletal health outcomes. Several systematic reviews of the literature linking *VDR* polymorphisms to various disease outcomes have been performed to date [3–7]. Reviews of studies investigating the influence of variation in other vitamin D pathway genes on bone health have also been performed [8]. However, reviews of studies that have investigated associations with non-skeletal health and variants in vitamin D pathway genes other than VDR are lacking. This is a significant omission, because genome-wide association studies have reported that polymorphisms in the genes encoding enzymes responsible for both synthesis and catabolism of 25-hydroxyvitamin D influence vitamin D status [9,10]. Such variants might therefore be expected to influence non-skeletal health outcomes in their own right, or to modify the effects of vitamin D supplementation on risk of extra-skeletal disease—a hypothesis that we have addressed in clinical trials [11,12].

We therefore conducted a literature review to identify genetic association studies reporting positive associations between risks of non-skeletal disease outcomes and single nucleotide polymorphisms (SNP) in the following genes encoding key players in the vitamin D pathway: *DHCR7*, *CYP2R1*, *CYP3A4*, *CYP27A1*, *DBP*, *LRP2*, *CUBN*, *CYP27B1*, *CYP24A1*, *VDR* and *RXRA*. The role for each of these genes in the vitamin D metabolic, transport and signaling pathways is illustrated in Fig. 1.

2. Methods

2.1. Search method

To identify eligible studies we searched the Pubmed database using the following terms: 'DHCR7'; 'CYP2R1'; 'CYP3A4'; 'CYP27A1'; 'DBP'; 'LRP2'; 'Megalin'; 'CUBN'; 'Cubilin'; 'CYP27B1'; 'CYP24A1'; 'VDR'; 'RXRA'. Our initial search was conducted in April of 2012 and captured manuscripts published from 2000 to 2012; we then conducted the same search in June of 2015 to capture manuscripts published from 2012 to 2015. Abstracts and titles were reviewed to select studies on the basis of inclusion / exclusion criteria below. All articles were assessed for eligibility by one author (DAJ); those selected for inclusion were re-assessed by a second (ARM).

2.2. Inclusion/exclusion criteria

2.2.1. Inclusion criteria

Candidate and genome-wide association studies in which SNP in the genes above were reported to associated with:

- Circulating concentrations of 25-hydroxyvitamin D
- Circulating concentrations of 1,25-dihydroxyvitamin D
- Susceptibility to, severity of, or prognosis of any non-skeletal health outcome.

2.2.2. Exclusion criteria

- Studies in which SNP in the above genes were reported to be associated with skeletal health outcomes
- Studies which investigated associations between a given polymorphism and a given health outcome which had been previously reviewed in a meta-analysis. In which case, we reviewed the meta-analysis instead.

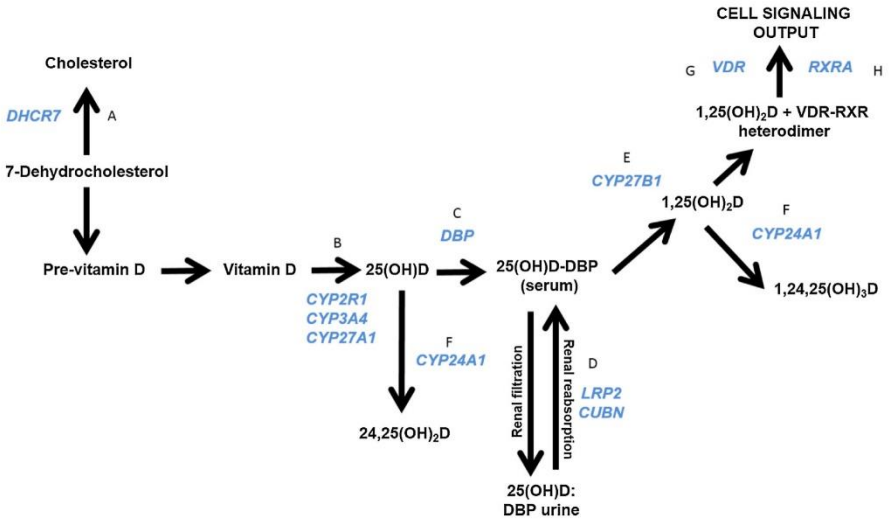

Fig. 1. A diagram depicting vitamin D metabolic and signalling pathways and genes encoding key players (in blue): *DHCR7* (A) encodes the 7-dehydrocholesterol reductase enzyme, which catalyses the conversion of 7-dehydrocholesterol to cholesterol; *CYP2R1*, *CYP3A4*, and *CYP27A1* (B) encode 25-hydroxylating cytochrome P450 enzymes; the vitamin D binding protein gene (*DBP*, [C]) encodes the principle vitamin D transport protein; *LRP2* and *CUBN* (D) encode the proteins megalin and cubilin, respectively, involved in renal re-absorption of 25(OH)D via receptor-mediated endocytosis; *CYP27B1* (E) encodes the cytochrome P450 enzyme which 1-alpha-hydroxylates 25(OH)D to form $1,25(OH)_2D$; *CYP24A1* (F) encodes the cytochrome P450 enzyme responsible for 24-hydroxylating vitamin D metabolites including 25(OH)D and $1,25(OH)_2D$; *VDR* (G) encodes the vitamin D receptor, which binds $1,25(OH)_2D$ and forms a heterodimer with the gene product of *RXRA* (H)—the retinoid X receptor—to mediate the biological actions of vitamin D. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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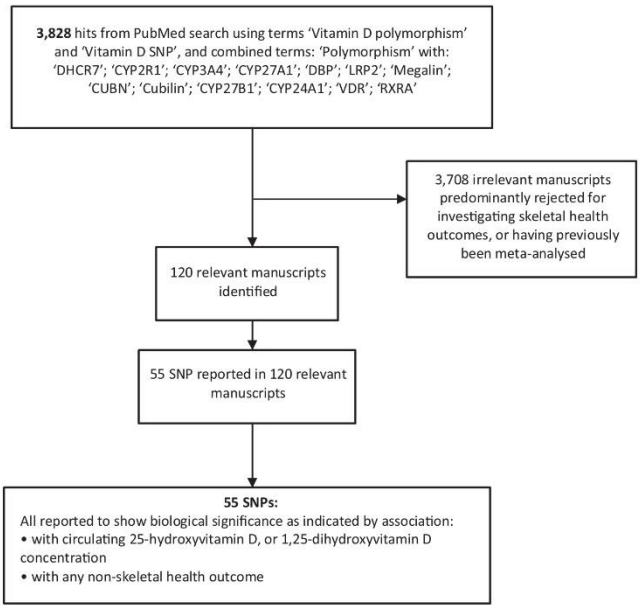

Fig. 2. Flow diagram depicting the literature search and SNP selection process.

3. Results

3.1. Identification and selection of studies

Fig. 2 depicts the study selection process. Our initial search identified 3828 publications of which 120, containing a total of 55 individual SNP, met eligibility criteria.

3.2. Study characteristics

Of the 120 studies selected for inclusion, 44 studies reported a combined 114 findings of significant association between the concentration of 25-hydroxyvitamin D or 1,25-dihydroxyvitamin D and genotype of 35 vitamin D pathway SNP. Of these 35 SNP, 13 were in *DBP*; 7 in *CYP2R1*; 7 in *DHCR7*; 4 in *CYP27B1*; 2 in *CYP24A1*; 1 in *RXRA*; and 1 in *VDR*. The remaining 76 studies reported a combined 105 reports of significant association between non-skeletal health outcomes (50 different diseases) and genotype of 29 vitamin D pathway SNP. Of these 29 SNP, 12 were in *VDR*; 4 in *DBP*; 3 in *CYP24A1*; 3 in *CYP27B1*; and 1 in each of: *CYP2R1*, *CYP3A4*, *CYP27A1*, *DHCR7*, *LRP2*, *CUBN* and *RXRA*. 11 SNP associated with both vitamin D metabolite concentration and susceptibility to non-skeletal disease.

3.3. Study findings

Table 1 presents results of 44 studies to have reported at least one association between a SNP in the vitamin D pathway and vitamin D metabolite concentrations: the majority of findings relate to variation in *DBP* gene, though a significant number were also identified for *CYP2R1* and *DHCR7* genes. Tables 2 and 3 present results from 76 studies to have reported at least one association between a SNP in the vitamin D pathway and susceptibility to non-skeletal health outcomes: variation in *VDR* represents the bulk of the findings. Of note, four of the most commonly investigated *VDR* polymorphisms (*FokI*, *ApaI*, *BsmI* and *TaqI*) account for 64% of the identified associations. Of the fifty different disease states identified as being associated with genetic variation in the vitamin D pathway, infectious and auto-immune diseases represent the most reported category (24/50), followed by cancers (12/50).

4. Discussion

To our knowledge, this is the first review to synthesise the literature reporting positive associations between genetic variation in the vitamin D pathway as a whole and biochemical and non-skeletal health outcomes. As might be expected, mutations in *DBP*, which encodes the binding protein that maintains serum concentration of 25(OH)D, is the most widely investigated source of variation in circulating concentrations of vitamin D metabolites. Mutations in *CYP2R1* and *DHCR7*, Table 3 which encode the major enzymes 'upstream' of 25(OH)D, were also consistently shown to be determinants of vitamin D status. This review also highlights a considerable number of SNP within these and other vitamin D pathway genes that were not identified as determinants of vitamin D status by the major GWAS. In many cases they have been validated and remain significant in different ethnic groups: this highlights the value of candidate-gene approaches in identifying rarer SNP whose effects may not be detected in genome-wide studies. A large number of studies (76—of which 19 were meta-analyses) reported associations between genetic variation in the vitamin D pathway and susceptibility, severity, or prognosis of extra-skeletal disease. The largest disease group for which such associations were reported are infectious and auto-immune related disorders (48%): this finding highlights the growing appreciation of the immunomodulatory effects of vitamin D and their importance for human health. The observation that there is limited overlap between genetic determinants of vitamin D status

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Table 1
Single nucleotide polymorphisms in vitamin D metabolic, transport and signalling pathways reported to associate with 25-hydroxyvitamin D (25D) and/or 1,25-dihydroxyvitamin D (1,25D) concentrations.

Gene (location)	SNP	SNP location/description	Reference	Findings/omments
VDR (chr12q13.11)	**rs10783219**	Intron 0, A > T	1. Engelman et al. [13] 2. Lee et al. [14]	1. Associates with 25D levels in San Antonio Hispanics ($P = 0.004$); not San Luis Valley Hispanics, or Los Angeles African Americans 2. Associates with 25D levels in largely white cohort: TA more frequent in deficient group (63%); AA more frequent in replete group (70%), $P = 0.013$
DBP (chr4q13.3)	**rs4588**	Exon 11, C > A. Defines Gc phenotype with rs7041	1. Lauridsen et al. [15] 2. Kurylowicz et al. [16] 3. Wjst et al. [17] 4. Engelman et al. [13] 5. Abbas et al. [18] 6. Fu et al. [19] 7. Sinotte et al. [20] 8. Fang et al. [21] 9. Janssens et al. [22] 10. Ahn et al. [10] 11. Robien et al. [23]	1–11. Minor allele (A) consistently associates with lower 25D/1,25D levels. rs4588-rs7041 haplotype: Gc1s-1s (CC rs4588; GG rs7041) associates with higher 25D status; Gc2-2 (AA rs4588; TT rs7041) associates with lower 25D status; Gc1s-1s (CC rs4588; GG rs7041) associates with higher 1,25D concentration; Gc2-2 (AA rs4588; TT rs7041) associates with lower 1,25D concentration
	rs7041	Exon 11, T > G. Defines Gc phenotype with rs4588	1. Lauridsen et al. [15] 2. Abbas et al. [18] 3. Sinotte et al. [20] 4. Fang et al. [21] 5. Janssens et al. [22] 6. Ahn et al. [10] 7. Wang et al. [9] 8. Robien et al. [23] 9. Engelman et al. [13]	1–9. Minor allele (G) consistently associates with higher 25D/1,25D levels. rs4588-rs7041 haplotype: Gc1s-1s (CC rs4588; GG rs7041) associates with higher 25D status; Gc2-2 (AA rs4588; TT rs7041) associates with lower 25D status; Gc1s-1s (CC rs4588; GG rs7041) associates with higher 1,25D concentration; Gc2-2 (AA rs4588; TT rs7041) associates with lower 1,25D concentration
	rs1155563	Intron 1, T > C	1. Ahn et al. [10] 2. Hibler et al. [24] 3. Lu et al. [25] 4. Zhang et al. [26] 5. Perna et al. [27] 6. Suaini et al. [28] 7. Elkum et al. [29] 8. Anderson et al. [30]	1 and 2. GWAS HIT: associates with 25D levels in predominantly white participants. In high LD with rs2282679 and rs7041 3 and 4. Haplotypes including rs1155563 associates with 25D levels in Chinese cohorts 5. Season-specific association with 25D levels in German older adults. 6. Minor allele associates with greater odds of vitamin D insufficiency ($\leq$50 nmol/L) in 12 mo Caucasian infants 7. Associates with 25D levels in Arab ($P = 0.03$), but not South Asian or Southeast Asian participants 8. GWAS in children: associates with 25D levels in those aged 14 yrs old
	rs17467825	3′UTR, A > G	1. Wang et al. [9] 2. Suaini et al. [28] 3. Elkum et al. [29] 4. Anderson et al. [30] 5. Nissen et al. [31]	1. GWAS HIT associates with 25D levels; replicated. Overall $P = 6.75 \times 10^{-74}$ 2. Minor allele associates with greater odds of vitamin D insufficiency ($\leq$50 nmol/L) in 12 mo Caucasian infants 3. Associates with 25D levels in Arab ($P = 0.02$) and South Asian participants ($P = 0.001$), but not Southeast Asians 4. GWAS in children: associates with 25D levels in 6 yr olds 5. Associates with 25D levels in Danish children and adults ($P < 0.0001$): GG (Minor hom) −13.7 nmol/L vs. AA
	rs2070741	A > C	Wood et al. [32]	Minor allele (C) allele mildly associates with decreased 25D levels in Caucasians ($P = 0.05$)
	rs2298849	Intron 1, T > C	1. Signorello et al. [33] 2. Robien et al. [23] 3. Xu et al. [34]	1. Associates with 25D levels in African Americans ($P = 0.01$): AA (Minor hom) −8.2 nmol/L vs. GG. No association in Caucasian participants 2. Associates with 25D levels in Chinese adults ($P = 0.001$): CC (Min hom) +8.3 nmol/L vs. TT 3. Associates with 25D levels in post-menopausal Chinese Hans ($P < 0.001$)
	rs16846876	A > T	1. Hibler et al. [24] 2. Nissen et al. [31]	1. Associates with 25D levels. Mean change under additive model: −2.95 (units not reported) (95% CI −4.27 to −1.63, $P < 0.001$) 2. Associates with 25D levels in Danish children and adults ($P < 0.001$): TT (Minor hom) −11.2 nmol/L vs. AA
	rs842999	G > C	1. Hibler et al. [15] 2. Nissen et al. [31]	1. Associates with 25D levels. Mean change under additive model: −1.92 (units not reported) (95% CI −3.10 to −0.73, $P = 0.002$) 2. Associates with 25D levels in Danish children and adults ($P < 0.001$): CC (Minor hom) −11.1 nmol/L vs. GG
	rs222035	Intron 8, A > C	Hibler et al. [24]	Associates with 25D levels. Mean change under additive model: −2.21 (units not reported) (95% CI −3.46 to −0.95, $P = 0.001$)
	rs3755967	G > A	1. Wang et al. [9] 2. Suaini et al. [28] 3. Elkum et al. [29]	1. GWAS HIT: associates with 25D levels; replicated. Overall $P = 2.42 \times 10^{-75}$ 2. Minor allele associates with greater odds of vitamin D insufficiency ($\leq$50 nmol/L) in 12 mo Caucasian infants 3. Associates with 25D levels in Arab ($P = 0.04$) and South Asian participants ($P = 0.0007$), but not Southeast Asians
	rs2298850	G > C	1. Wang et al. [9] 2. Elkum et al. [29]	1. GWAS HIT: associates with vitamin D levels; replicated. Overall $P = 2.03 \times 10^{-71}$ 2. Associates with 25D levels in Arab ($P = 0.04$) and South Asian participants ($P = 0.01$), but not Southeast Asians. In high LD ($r^2 > 0.8$) with rs4588
	rs12512631	3′UTR, T > C	1. Ahn et al. [4] 2. Perna et al. [27]	1. Associates with 25D levels ($P = 0.0004$): CC (Minor hom) +8.2 nmol/L vs. TT

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Table 1 (*Continued*)

Gene (location)	SNP	SNP location/description	Reference	Findings/omments
			3. Nissen et al. [31]	2. Season-specific association with 25D levels in German older adults
			4. Barry et al. [35]	3. Associates with 25D levels in Danish children and adults
				4. Associates with 25D levels in White adults, US ($P < 0.0001$)
	rs2282679	Intron 12, A > C	1. Ahn et al. [4]	1. Associates with 25D levels ($P = 0.00004$): CC (Minor hom) −6.6 nmol/L vs. AA
			2. Wang et al. [9]	2. GWAS hit: strong association with 25D levels ($P = 1.9 \times 10^{-109}$); is in LD with rs7041 and rs1155563
			3. Signorello et al. [33]	3. Associates with 25D levels in African Americans ($P = 0.03$): GG (Minor hom) −8.3 nmol/L vs. TT. No association in Caucasian participants
			4. Perna et al. [27]	4. Season-specific association with 25D levels in German older adults
			5. Cheung et al. [36]	5. Minor allele associated with greater odds of vitamin D insufficiency (≤50 nmol/L) in adult Chinese females: OR 1.51 (95% CI 1.19–1.93, $P = 8.6 \times 10^{-4}$)
			6. Suaini et al. [28]	6. Minor allele associates with greater odds of vitamin D insufficiency in 12 mo Caucasian infants
			7. Elkum et al. [29]	7. Associates with 25D levels in Arab and South Asian participants
			8. Nissen et al. [31]	8. Associates with 25D levels in Danish children and adults
CYP2R1 (chr11p15.2)	**rs10741657**	G > A	1. Ramos-Lopez et al. [37]	1. Associates with 25D levels ($P = 0.003$)
			2. Wang et al. [9]	2. GWAS Hit: associates with 25D levels; replicated (Overall $P = 3.3 \times 10^{-20}$)
			3. Robien et al. [23]	3. Associates with 25D levels in Chinese adults ($P = 0.2$): AA (Min hom) +5.1 nmol/L vs. GG
			4. Hassanein et al. [38]	4. Associates with 25D levels in males
			5. Barry et al. [35]	5. Associates with 25D levels in White adults, US ($P < 0.0001$)
			6. Batai et al. [39]	6. Minor allele (A) associates with increased 25D levels in African Americans ($P = 0.01$) and European Americans (0.003)
			7. Nissen et al. [31]	7. Associates with 25D levels in Danish children and adults ($P < 0.0001$): AA (Min hom) +9.4 nmol/L vs. GG
			8. Ye et al. [40]	8. Associates with 25D levels in large combined dataset of participants of European descent: −3.22 nmol/L per risk allele (95% CI 1.79– 4 × 66, $P < 0.05$)
	rs2060793	5′UTR, G > A	1. Ahn et al. [10]	1. GWAS Meta-analysis: associates with 25D levels (Overall $P = 1.4 \times 10^{-5}$)
			2. Zhang et al. [26]	2. Associated with 25D levels in Chinese Hans (9.4×10^{-14}). In perfect LD ($r^2 = 1.0$) with rs10741657
	rs1993116	Intron 1, C > T	1. Wang et al. [9]	1 and 2. GWAS HIT: associates with 25D levels (Overall $P = 6.25 \times 10^{-11}$ in Wang, 2010)
			2. Ahn et al. [10]	
			3. Robien et al. [23]	3. Associates with 25D levels in Chinese adults ($P = 0.4$): TT (Min hom) +3.9 nmol/L vs. CC
			4. Batai et al. [39]	4. Minor allele (T) associates with increased 25D levels in African Americans ($P = 0.02$) and European Americans (0.0006)
	rs7116978	C > T	1. Wang et al. [9]	1. GWAS HIT: associates with 25D levels (Overall $P = 4.99 \times 10^{-9}$)
			2. Nissen et al. [31]	2. Associates with 25D levels in Danish children and adults ($P < 0.0001$): TT (Min hom) +10.7 nmol/L vs. CC
	rs12794714	Exon 1,G > A	1. Wang et al. [9]	1. GWAS HIT: associates with 25D levels (Overall $P = 1.84 \times 10^{-9}$)
			2. Robien et al. [23]	2. Associates with 25D levels in Chinese adults ($P < 0.001$): AA (Min hom) −10.6 nmol/L vs. GG
			3. Barry et al. [35]	3. Associates with 25D levels in White adults, US ($P < 0.0001$)
			4. Batai et al. [39]	4. Minor allele (A) associates with decreased 25D levels in African Americans ($P = 0.01$) and European Americans ($P = 0.005$)
	rs10500804	A > C	1. Wang et al. [9]	1. GWAS HIT: associates with 25D levels (Overall $P = 2.67 \times 10^{-9}$)
			2. Azad et al. [41]	2. CC (Min hom) associates with lower 25D levels vs. AA in Canadians ($P = 0.001$)
			3. Elkum et al. [29]	3. Associates with 25D levels in Arab ($P = 0.04$), but not South Asian, or South East Asian participants
	rs10766197	G > A	1. Zhang et al. [26]	1. Associates with 25D levels in Chinese Hans ($P = 0.004$)
			2. Barry et al. [35]	2. Associates with 25D levels ($P = 0.0002$) and differential response to vitamin D supplementation ($P = 0.02$) in White adults, US
			3. Nissen et al. [31]	3. Associates with 25D levels in Danish children and adults ($P < 0.0001$): AA (Min hom) −7.4 nmol/L vs. GG
CYP24A1 (chr20q13.2)	**rs6013897**	G > A	1. Wang et al. [9]	1. GWAS HIT: associates with 25D levels (Overall $P = 6.0 \times 10^{-10}$)
			2. Cooper et al. [42]	2. Associates with 25D levels in UKBS-CC cohort ($P = 0.02$)
			3. Barry et al. [35]	3. Associates with differential response to vitamin D supplementation in White adults, US ($P = 0.04$)
	rs2248137	C > G	Pillai et al. [43]	Minor allele associates with decreased 25D levels ($P = 0.006$)
CYP27B1 (chr12q14.1)	**rs10877012**	Promoter region, C > A	1. Hyppönen et al. [44]	1. Associates with 25D levels: 1.9% difference for A vs. C allele ($P = 0.01$)
			2. Signorello et al. [33]	2. Minor allele associates with higher 25D levels in African Americans; no association in Caucasians
			3. Lange et al. [45]	3. Associates with 1,25D levels: 72, 61, and 60 pmol/ml for AA, AC, and CC, respectively ($P = 0.04$)
	rs118204009	C > T	Ramagopalan et al. [46]	Associates with 1,25D levels. Causes complete loss of CYP27B1 function
	rs703842	5′ UTR, T > C	Orton et al. [47]	Associates with 25D levels in Canadian twins ($P < 0.001$): TT genotype +27 nmol/L vs. CC genotype
	rs4646536	Intron 6, T > C	Orton et al. [47]	Associates with 25D levels in Canadian twins ($P < 0.001$): TT genotype +24 nmol/L vs. CC genotype
DHCR7 (chr11q13.4)	**rs12785878**	Intron 2, G > T	1. Wang et al. [9]	1. GWAS Hit: associates with 25D levels; replicated (Overall $P = 2.12 \times 10^{-27}$)
			2. Zhang et al. [2,48]	
			3. Cheung et al. [36]	2. Associated with 25D levels in Chinese Han children ($P = 0.01$)

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Table 1 (*Continued*)

Gene (location)	SNP	SNP location/description	Reference	Findings/omments
			4. Strawbridge et al. [49] 5. Voipio et al. [50]	3. Associated with vitamin D insufficiency (<50 nmol/L) in Southern Chinese women ($P = 0.04$) 4. G allele associates with decreased 25D levels ($P = 0.0006$) 5. G allele associates with increased odds of vitamin D insufficiency (<50 nmol/L) in Finnish adults: OR 1.31 (95% CI 1.00–1.70, $P < 0.05$)vs. T allele
	rs3829251	G > A	1. Ahn et al. [10] 2. Lu et al. [25] 3. Zhang et al. [2,48] 4. Strawbridge et al. [49]	1. GWAS Meta-analysis: associates with 25D levels (Overall $P = 8.8 \times 10^{-7}$) 2. Risk alleles (AG) for rs3829251 and rs1790349 associate with decreased 25D levels in Chinese Han adults 3. Associated with 25D levels in Chinese Han children ($P = 0.001$) 4. Minor allele (A) associates with decreased 25D levels ($P = 0.0004$)
	rs7944926	Intron 1, G > A	1. Wang et al. [9] 2. Davies et al. [51]	1. GWAS Hit: associates with 25D levels; replicated (Overall $P = 8.96 \times 10^{-16}$) 2. Associates with 25D levels in UK participants: AA genotype-6.0 nmol/L vs. GG ($P = 0.03$)
	rs12800438	G > A	1. Wang et al. [9] 2. Batai et al. [39]	1. GWAS Hit: associates with 25D levels; replicated (Overall $P = 2.54 \times 10^{-15}$) 2. Risk genotype associated with increased odds of vitamin D insufficiency (<50 nmol/L) in African Americans: OR 0.76 (95% CI 0.58–0.99, $P = 0.04$). In perfect LD with rs12785878 ($r^2 = 1.0$)
	rs3794060	C > T	Wang et al. [9]	GWAS Hit: associates with 25D levels; replicated (Overall $P = 3.38 \times 10^{-15}$). In perfect LD with rs12785878 ($r^2 = 1.0$)
	rs4945008	G > A	Wang et al. [9]	GWAS Hit: associates with 25D levels; replicated (Overall $P = 4.55 \times 10^{-15}$). In high LD with rs12785878 ($r^2 = 0.95$)
	rs4944957	G > A	Wang et al. [9]	GWAS Hit: associates with 25D levels; replicated (Overall $P = 8.70 \times 10^{-15}$). In perfect LD with rs12785878 ($r^2 = 1.0$)
RXRA (chr9q34.3)	**rs9409929**	G > A	Hibler et al. [52]	Associates with 1,25D levels: increasing concentration with increasing no. copies of the A allele (P-trend = 0.003)

Abbreviations: CI: confidence interval; Min hom: minor homozygous; VDR: vitamin D receptor, DBP: vitamin D binding protein, Gc: group-specific component; GWAS: genome-wide association study, LD: linkage disequilibrium, 3'UTR: 3-prime untranslated region, CYP2R1: cytochrome P450-2R1, 5'UTR: 5-prime untranslated region, UKBS-CC: U.K. Blood Services Common Controls, CYP24A1: cytochrome P450-24A1, CYP27B1: cytochrome P450-27B1, DHCR7: 7-dehydrocholesterol reductase, RXRA: retinoid-X receptor A.

Table 2
Single nucleotide polymorphisms in the vitamin D signalling pathway, reported to associate with non-skeletal health outcomes.

Gene	SNP (description)	Disease association	Reference	Findings/comments
VDR (chr12q13.11)	**rs10735810** (Exon 2, C[F] > T [f], *FokI* restriction endonuclease. Previously rs2228570)	Severe RSV infection	Kresfelder et al. [53]	f allele associates with increased risk in South African children: OR 1.820 (95% CI 1.183–2.801, $P = 0.006$) vs. F allele
		Tuberculosis	1. Wilkinson et al. [54] 2. Zhang et al. [55] 3. Sharma et al. [56]	1. ff genotype or undetectable serum 25D levels associate with increased risk in Gujarati Asians living in West London: OR 5.1 (95% CI 1.4–18.4, $P = 0.008$) vs. FF 2. ff genotype associates with increased spinal TB susceptibility in Chinese Hans: OR 2.18 (95% CI 1.24–3.83, $P < 0.05$) vs. FF 3. ff genotype associates with increased risk of MDR smear +ve TB in North Central Indian castes: OR 3.4 ($P = 0.01$) vs. FF
		Psoriasis	Lee et al. [57]	Meta-analysis: ff genotype associates with increased risk in Turkish cohorts: OR 3.58 (95% CI 1.60–8.01, $P = 0.002$) vs. FF
		Hepatitis B	Li et al. [58]	fF/ff genotypes associate with increased risk: OR 1.70 ($P = 0.02$) vs. FF
		Acute lower respiratory infection	Roth et al. [59]	ff genotype associates with increased risk in early childhood: OR 7.38 (95% CI 1.17–46.55, $P = 0.03$) vs. FF
		Urinary tract infection	Aslan et al. [60]	ff genotype associates with increased risk in children: OR 3.94 (95% CI 1.71–9.09, $P < 0.01$) vs. FF
		Asthma	1. Pillai et al. [43] 2. Nabih and Kamel [61]	1. Associates with IgE concentration ($P = 0.002$); pre-bronchodilator and change in FEV_1/FVC ($P = 0.02$), in young African Americans 2. ff genotype associates with increased IgE and Th2 cytokine concentrations in Egyptian children ($P = 0.007$)
		Hypertension	Swapna et al. [62]	FF genotype associates with increased risk: OR 1.20 (95% CI 1.23–3.93, $P < 0.01$) vs. ff
		Systemic lupus erythematosus	Luo et al. [63]	F allele associates with increased risk: RR 1.630 (95% CI 1.21–2.20, $P = 0.001$) vs. f allele
		Renal cell carcinoma	Arjumand et al. [64]	ff genotype combined with bb genotype for BsmI associates with increased risk in North Indians: OR 1.88 (95% CI 1.05–3.63, $P = 0.04$) vs. FF + BB genotypes
		Rheumatoid arthritis	Lee et al. [65]	Meta-analysis: F allele associates with increased risk in Europeans: OR 1.50 (95% CI 1.16–1.95, $P = 0.002$) vs. f allele
		Breast cancer	Mun et al. [66]	Meta-analysis: ff genotype associates with increased risk ($P < 0.05$) vs. FF

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Table 2 (*Continued*)

Gene	SNP (description)	Disease association	Reference	Findings/comments
		Thyroid cancer	Penna-Martinez et al. [67]	ff genotype associates with increased risk in Germans ($P = 0.04$) vs. FF
		Diabetes (type 1)	Panierakis et al. [68]	F allele associates with decreased risk in Cretan Greeks: OR 0.52 (95% CI 0.32–0.85, $P = 0.008$) vs. f allele
		Ovarian cancer	Mun et al. [66]	Meta-analysis: ff genotype associates with increased risk ($P < 0.05$) vs. FF
		RSV-related disease	Kresfelder et al.[53]	Associates with risk in South African children ($P = 0.008$)
		Parkinson's disease	Gao et al. [69]	Meta-analysis: f allele associates with increased risk: OR 1.41 (95% CI 1.14–1.75, $P = 0.001$) vs. F allele
		S. aureus carriage	Messaritakis et al. [70]	ff genotype associates with increased risk in Cretan Greeks with Type 2 Diabetes ($P < 0.001$) vs. FF
		Diabetic retinopathy	Zhong et al. [71]	f allele associates with increased risk in Chinese Hans with Type 2 Diabetes: OR 1.47 ($P < 0.01$) vs. F allele
	rs1544410 (Intron 8, A [B] > G[b], *BsmI* restriction endonuclease. In high LD [r^2=0.9] with rs731236)	HIV	1. Moodley et al. [72] 2. Torres et al. [73]	1. BB genotype associates with more rapid disease progression in Hispanic children: HR 6.60($P = 0.03$) vs. bb 2. bb genotype associates with delayed progression to AIDS and increased resistance to HIV-1
		Psoriasis	Lee et al. [57]	Meta-analysis: B allele associates with decreased risk in Asians: OR 0.64 (95% CI 0.41–0.98, $P = 0.04$) vs. b allele
		Autoimmune thyroid diseases	Feng et al. [74]	Meta-analysis: B allele associates with decreased risk of autoimmune thyroid diseases (Graves' disease & Hashimoto's thyroiditis): OR 0.80 (95% CI 0.71–0.91, $P = 0.001$) vs. b allele
		Tuberculosis	Sharma et al. [56]	bb genotype associates with risk of smear +ve & MDR TB in three Central India populations: Tribes (OR 3.7, $P = 0.002$); South Eastern-Castes (OR 2.1, $P = 0.0004$); and Muslims (OR 6.7 $P = 0.01$) vs. BB
		SLE and lupus nephritis	Lee et al. [65]	Meta-analysis: B allele associates with increased risk of SLE (OR 3.58, 95% CI 1.41–9.13, $P = 0.007$) and LN (OR 3.65, 95% CI 1.35–9.90, $P = 0.011$) in Asians, vs. b allele
		End-stage renal disease	Testa et al. [75]	Associates with risk of ESRD, as measured by left-ventricular mass index ($P = 0.006$)
		Diabetes (type 1)	Panierakis et al. [68]	B allele associates with decreased risk in Cretan Greeks: OR 0.65 (95% CI 0.44–0.97, $P = 0.04$) vs. b allele
		Osteoporosis	Jia et al. [76]	Meta-analysis: bb genotype associates with decreased risk across 26 studies: OR 0.61 (95% CI 0.40–0.92, $P < 0.05$) vs. BB
		Colorectal cancer	Jenab et al. [77]	BB genotype associates with decreased risk in Europeans: RR, 0.76 (95% CI 0.59–0.98, $P < 0.05$) vs. bb
		Melanoma	Orlow et al. [78]	bb genotype associates with increased risk in multi-centre study; predominantly Caucasians: OR 1.30 (95% CI 1.04–1.63, $P < 0.05$) vs. BB
		Periodontitis	Deng et al. [79]	Meta-analysis: bb genotype associates with decreased risk in Asians: OR 0.63 (95% CI 0.42–0.94, $P = 0.02$) vs. BB
		Vitiligo	Li et al. [80]	Meta-analysis: bb genotype associates with increased risk in East Asians: OR 1.32 (95% CI 1.09–1.59, $P < 0.01$) vs. BB
		Renal cell carcinoma	Ou et al. [81]	Meta-analysis: BB genotype associates with risk in Asians ($P < 0.05$) vs. bb
		Gout	Liu et al. [82]	b allele associates with increased risk in male Chinese Hans: OR 1.57 (95% CI 1.14–2.18, $P = 0.006$) vs. B allele
	rs7975232 (Intron 8, A [A] > C[a], *ApaI* restriction endonuclease)	HAM/TSP	Saito et al. [83]	AA genotype associates with reduced risk of HAM/TSP: OR 0.28 (95% CI 0.13–0.63, $P = 0.001$) vs. aa
		Colorectal cancer	Mahmoudi et al.[84]	aa genotype associates with increased risk of CRC in Iranians: OR 2.32 (95% CI 1.19–4.54, $P = 0.014$) vs. AA
		Graves' disease	Abd El Gawad et al. [85]	aa genotype associates with increased risk of GD: OR 2.79 (95% CI 1.12–6.93, $P < 0.05$) vs. AA
		Psoriasis	Lee et al. [57]	Meta-analysis: A allele associates with decreased risk of psoriasis in Turkish populations: OR 0.68 (95% CI 0.48–0.99, $P = 0.04$) vs. a allele
		Asthma	Saadi et al. [86]	AA genotype associates with increased risk of asthma in Chinese Han population: OR 1.33 (95% CI 1.10–1.60, $P = 0.002$) vs. aa
		Thyroid cancer	Penna-Martinez et al. [67]	aa genotype associates with increased risk in Germans ($P = 0.04$) vs. AA
		Diabetes (type 1)	Panierakis et al. [68]	A allele associates with increased risk in Cretan Greeks: OR 1.61 (95% CI 1.07–2.41, $P = 0.02$) vs. a allele
		Prostate cancer	1. Onen et al. [87] 2. Jingwi et al. [88]	1. Associates with risk of sporadic PCa ($P = 0.009$). 2. Associates with PCa risk in African Americans ($P < 0.05$)
		Periodontitis	Deng et al. [79]	Meta-analysis: AA genotype associates with increased risk in Asians:OR 2.20 (95% CI 1.39–3.48, $P < 0.001$) vs. aa
		Hepatitis C	Baur et al. [89]	aa genotype associates with rapid fibrosis progression: OR 2.32 (95%CI 1.05–5.10, $P = 0.04$); increased risk of cirrhosis: OR 2.67 (95% CI 1.29–5.51, $P = 0.009$) vs. AA, in >90% Caucasian Swiss patients
		Atopic dermatitis	Heine et al. [90]	a allele associates with increased risk: OR 1.57 (95% CI 1.10–1.96, $P = 0.006$) vs. A allele
		Breast cancer	Dalessandri et al. [91]	aa genotype associates with increased risk ($P = 0.0003$) vs. AA genotype
		Hepatocellular carcinoma	Hung et al. [92]	aa genotype associates with increased development: OR 3.02 (95% CI 1.65–5.51, $P < 0.05$) vs. AA

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Table 2 (*Continued*)

Gene	SNP (description)	Disease association	Reference	Findings/comments
		Leprosy	Neela et al. [93]	A allele associates with increased risk in Indians ($P = 0.001$) vs. a allele
		Vitiligo	Li et al. [80]	Meta-analysis: aa/aA genotypes associate with increased risk in East Asians: OR 1.40 (95% CI 1.01–1.96, $P < 0.05$) vs. AA
		Renal cell carcinoma	Ou et al. [81]	Meta-analysis: AA genotype associates with risk in Asians ($P < 0.05$) vs. aa
		Dengue fever	Alagarasu et al. [94]	a allele associates with decreased risk: OR 0.54 (95% CI 0.36–0.82, $P = 0.01$) vs. A allele
	rs731236 (Exon 9,T[T] > C [t], *TaqI* restriction endonuclease)	Oral squamous cell carcinoma	Bektas-Kayhan et al. [95]	Associates with risk ($P < 0.05$)
		Graves' Disease	Abd El Gawad et al. [85]	TT genotype associates with increased risk: OR 3.05 (95% CI 1.48–6.28, $P < 0.05$) vs. tt
		Diabetes (type 1)	Panierakis et al. [68]	T allele associates with increased risk in Cretan Greeks: OR 2.24 (95% CI 1.49–3.36, $P = 0.0001$) vs. t allele
		Autoimmune thyroid diseases	Feng et al. [74]	Meta-analysis: t allele associates with decreased risk: OR 0.85 (95% CI 0.76–0.96, $P = 0.01$) vs. T allele
		Systemic lupus erythematous	Carvalho et al. [96]	TT genotype associates with increased disease severity in North Portugese ($P = 0.046$) vs. tt
		Primary biliary cirrhosis	Li et al. [97]	T allele associates with decreased risk in Europeans and Asians: OR 0.75 (95% CI 0.63–0.89, $P = 0.001$) vs. t allele
		Breast cancer	Perna et al. [98]	tt genotype associates with increased risk of breast cancer mortality: HR 2.80 (95% CI 1.10–7.20, $P < 0.05$) vs. TT
		Obesity	Vasilopoulos et al. [99]	T allele associates with increased risk in Greeks: OR 2.07 (95% CI 1.12–3.82, $P = 0.02$) vs. t allele
		Periodontitis	Tanaka et al. [100]	tt genotype associates with increased risk in Japanese women: OR 3.68 (95% CI 1.06–12.78, $P < 0.05$) vs. TT
		Tuberculosis	Martineau et al. [11]	tt genotype associates with increased effect of vitamin D supplementation on sputum culture conversion time: 8.09 (95% CI 1.36–48.01, $P = 0.02$) vs. TT
		Multiple sclerosis	Agliardi et al. [101]	TT genotype associates with decreased risk in HLA-DRB1*15-positive MS patients: OR: 0.53 (95% CI 0.33–0.83, $P = 0.004$) vs. tt
	rs11568820 (Promoter, G > A, cdx2)	Rubella	Ovsyannikova et al. [102]	Minor allele (A) associates with decreased TNF-α concentration post rubella vaccination ($P = 0.02$) vs. G allele
		Alzheimer's disease	Wang et al. [103]	Minor allele (A) associates with increased risk of late-onset AD: OR 1.69 ($P = 9.1 \times 10^{-6}$) vs. G allele
		Gout	Liu et al. [82]	Minor allele (A) associates with increased risk in male Chinese Hans: OR 1.25 (95% CI 1.05–1.49, $P = 0.01$) vs. G allele
	rs7976091 (Promoter, C > T)	Alzheimer's disease	Wang et al. [103]	Minor allele (T) associates with increased risk of late-onset AD: OR 1.55 ($P = 8.9 \times 10^{-5}$) vs. C allele. In perfect LD ($r^2 = 1.0$) with rs11568820
	rs11574010	Multiple sclerosis	Dickinson et al. [104]	G allele associates with increased risk of MS in those with low sun exposure during childhood ($P = 0.01$)
	rs4516035	HIV	Torre et al. [105]	In a 5 SNP haplotype which associates with risk of HIV-1 infection: OR 0.4 (95% CI 0.22–0.72, $P = 0.003$)
	(EcoRV, A > G)	Non-Hodgkin lymphoma	Kelly et al. [106]	Associates with modified effect of early life sun exposure on risk of non-Hodgkin lymphoma P for interaction = 0.006)
		Melanoma	Orlow et al. [78]	GG genotype (min hom) associates with increased risk in predominantly Caucasian cohort: OR 1.25 (95% CI 1.01–1.55, $P = 0.05$) vs. AA
	rs7970314 (Promoter, A > G)	Rubella	Ovsyannikova et al. [102]	Minor allele (G) associates with decreased TNF-α concentration post rubella vaccination ($P = 0.03$) vs. A allele. In high LD ($r^2 = 0.92$) with rs11568820
		Parkinson's disease	Gao et al. [69]	Meta-analysis: Minor allele (G) associates with increased risk: OR 1.32 (95% CI 1.17–1.50, $P < 0.001$) vs. A allele
	rs2238136 (Intron 2, G > A)	Colorectal cancer	Mahmoudi et al. [84]	AA genotype associates with increased risk of colorectal cancer: OR 2.09 (95% CI 1.15–3.78, $P = 0.02$) vs. GG
		Melanoma	Kosiniak-Kamysz et al. [107]	GCCC haplotype for rs2238136-rs4516035-rs7139166-rs11568820 associates with increased risk: OR 5.65 (1.79–17.81, $P = 0.003$)
	rs2853559 (T/C)	Myopia	Mutti et al. [108]	T allele associates with increased risk of myopia in Caucasians: OR = 1.99 ($P = 0.003$)
	rs4334089 (G/A)	Parkinson's disease	Butler et al. [109]	Associates with age-at-onset ($P = 0.02$)
RXRA (chr9q34.3)	**rs7861779** (G > A)	Colorectal cancer	Jacobs et al. [110]	A allele associates with increased risk: OR 1.42 (95% CI 1.03–1.97, $P = 0.03$) vs. G allele

Abbreviations: VDR: vitamin D receptor, RSV: respiratory syncytial virus, OR: odds ratio, CI: confidence interval, TB: tuberculosis, MDR: multi-drug resistant, +ve: positive, IgE: Immunoglobulin E, Th2: T helper cell type-2, RR: risk ratio, PD: Parkinson's disease, S.aureus: Staphylococcus aureus, HIV: human immunodeficiency virus, LD: linkage disequilibrium, AIDS: acquired immune deficiency syndrome, AITD: autoimmune thyroid disease, SLE: systemic lupus erythematous, LN: lupus nephritis, HAM/TSP: HTLV-I (Human T-lymphotropic virus I)-associated myelopathy/tropical spastic paraparesis, PCa: prostate cancer, HR: hazard ratio, TNF-α: tumour necrosis factor-alpha, Cdx-2: caudal type homeobox-2, HIV-1: human immunodeficiency virus-1.

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Table 3
Single nucleotide polymorphisms in pathways of vitamin D metabolism and transport, reported to associate with non-skeletal health outcomes.

Gene	SNP (description)	Disease association	Reference	Findings/comments
DBP (chr4q13.3)	**rs7041/rs4588** (Exon 11, T > G; C > A)	Breast cancer	Anderson et al. [111]	TT genotype associates with increased risk of breast cancer in female Caucasians in Ontario: OR 1.23 (95% CI 1.01–1.51, $P < 0.05$) vs. GG
		Cancer	Jorde et al. [112]	Gc-1f/1f genotype associates with decreased risk in Norwegians: HR 0.74 (95% CI 0.59–0.93, $P = 0.008$), vs. Gc-2/2
		COPD	Janssens et al. [113]	TT genotype associates with increased risk in Caucasians: OR 2.11 (95% CI 1.20–3.71, $P = 0.009$) vs. GG
		HCC	Peng et al. [114]	G allele associates with increased risk of HCC in Chinese Han patients with HCV: OR 1.50 (95% CI 1.06–2.14, $P = 0.034$) vs. T allele
		Lung cancer	Kong et al. [115]	GG genotype associates with decreased risk: OR 0.57 (95% CI 0.35–0.93, $P < 0.001$) vs. TT
		Tuberculosis	Martineau et al. [116]	Gc-2/2 associates with increased risk in Gujarati Asians, with 25D levels <20 nmol/L: OR 2.81 (95% CI 1.19– 6.66, $P = 0.009$) vs. Gc-1/1
	rs1155563 (Intron 1, T > C)	COPD	Bakke et al. [117]	Associates with clinical marker of disease severity (FEV_1/FVC [$P < 0.05$])
	rs17467825 (3′UTR, A > G)	COPD	Bakke et al. [117]	Associates with clinical marker of disease severity (FEV_1 % predicted [$P < 0.05$]). In high LD with rs1155563 ($r^2 = 0.86$)
	rs2070741 (A > C)	Bronchiectasis/ COPD	Wood et al. [32]	C allele associates with increased risk of bronchiectasis: OR 1.80 (95% CI 1.0–3.19, $P = 0.03$) and increased risk of airway bacterial colonisation: OR 3.84 (95% CI 1.78–6.92, $P = 0.04$)
CYP2R1 (chr11p15.2)	**rs10766197** (G > A)	Asthma	Pillai et al. [43]	Associates with risk of asthma in young African Americans ($P = 0.04$)
CYP3A4 (chr7q21.1)	**rs2740574** (promoter, A > G, *CYP3A4*1B*)	Prostate cancer	1. Tayeb et al. [118] 2. Fernandez et al. [119]	1. GG genotype associates with increased risk of prostate cancer: RR 2.7 (95% CI 0.77–7.66) vs. AA 2. AG/GG genotypes associate with increased risk of prostate cancer in White and mixed ancestry South Africans: OR 3.27 (95% CI 2.30–4.65, $P < 0.004$) vs. AA
CYP27A1 (chr2q35)	**rs17470271** (A > T)	Asthma	Leung et al. [120]	Associates with clinical marker of asthma severity (FEV_1 [$P = 0.03$])
CYP24A1 (chr20q13.2)	**rs6127118** (Intron 7, G > A)	AMD	Morrison et al. [121]	Associates with risk of AMD in family cohort study ($P = 0.03$)
	rs2762934 (Exon 12, G > A)	AMD	Morrison et al. [121]	Associates with risk of AMD in family cohort study ($P = 0.01$)
		Breast cancer	Fuhrman et al. [122]	Minor allele associates with increased risk: OR 1.35 (95% CI 1.09–1.67, P for trend = 0.005), for each additional allele
	rs2762939 (Intron 5, G > A)	Coronary artery calcification	Shen et al. [123]	Meta-analysis: C allele associates with decreased risk in 3 studies (Overall $P = 2.9 \times 10^{-6}$)
CYP27B1 (chr12q14.1)	**rs10877012** (promoter, C > A,)	Autoimmune Addison's disease	Fichna et al. [124]	C allele associates with increased risk: OR 1.53 (95% CI 1.07–2.19, $P = 0.02$) vs. A allele
		Hepatitis C	Lange et al. [45]	C allele associates with reduced ability to achieve sustained virologic response ($P = 0.02$) vs. A allele
	rs4646536 (Intron 6, T > C,)	Diabetes (type 1)	Bailey et al. [125]	TT genotype associates with increased risk: OR 1.20 (95% CI 1.07–1.36, $P = 0.01$) vs. CC. In perfect ($r^2 = 1.0$) LD with rs10877012
		Congestive heart failure	Wilke et al. [126]	CC genotype associates with increased risk in patients with hypertension, of predominantly European ancestry: OR 2.14 (95% CI 1.05–4.39, $P < 0.05$), vs. TT
	rs4646537 (Intron 8, A > C)	Hypertension	Wilke et al. [126]	AC genotype associates with decreased risk in predominantly European ancestry participants: OR 0.35 (95% CI 0.13–0.91, $P < 0.05$) vs. AA
DHCR7 (chr11q13.4)	**rs12785878** (G > T)	Multiple sclerosis	Alloza et al. [127]	Associates with risk ($P < 0.01$)
LRP2 (chr4q35.1)	**rs3755166** (promoter, G > A)	Alzheimer's disease	1. Wang et al. [128] 2. Vargas et al. [129]	1. A allele associates with increased risk in Chinese Hans: OR 1.38 (95% CI 1.02–1.87, $P = 0.04$) vs. G allele 2. AA genotype associates with increased risk in Europeans without ApoE4 mutation: OR 1.41 (95% CI 1.10–1.90, $P = 0.03$) vs. GG
CUBN (chr10p12.31)	**rs3740165** (G > A)	Diabetes (type 1)	Ramos-Lopez et al. [130]	AA genotype associates with increased risk ($P = 4 \times 10^{-7}$) vs. GG
RXRA (chr9q34.3)	**rs7861779** (G > A)	Colorectal cancer	Jacobs et al. [110]	A allele associates with increased risk: OR 1.42 (95% CI 1.03–1.97, $P = 0.03$) vs. G allele

Abbreviations: DBP: vitamin D binding protein, CYP2R1: cytochrome P450-2R1, CYP3A4: cytochrome P450-3A4, CYP27A1: cytochrome P450-27A1, CYP24A1: cytochrome P450-24A1, CYP27B1: cytochrome P450-27B1, DHCR7: 7-dehydrocholesterol reductase, LRP2: lipoprotein receptor-related protein 2 (Megalin), CUBN: cubilin, OR: odds ratio, CI: confidence interval, COPD: chronic obstructive pulmonary disease, HCC: hepatocellular carcinoma, HCV: hepatitis C virus, LD: linkage disequilibrium, FEV_1: forced expiratory volume in 1 s, FVC: forced vital capacity, AMD: age-related macular degeneration.

and those associated with non-skeletal health outcomes is striking. This may reflect the relative lack of studies investigating influence of variation in genes other than VDR on health outcomes; alternatively it may have biological significance, suggesting that variation in VDR is a more important determinant of phenotype than circulating 25(OH)D concentrations.

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Review

Environmental and genetic determinants of vitamin D status among older adults in London, UK

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ABSTRACT

Despite the high prevalence of vitamin D deficiency among older adults in the UK, studies investigating the determinants of vitamin D status in this group are lacking. We conducted a cross-sectional study in 222 older adults living in sheltered accommodation in London, UK, who were screened for participation in a clinical trial of vitamin D supplementation for the prevention of acute respiratory infection. Details of potential demographic and lifestyle determinants of vitamin D status were collected by questionnaire and blood samples were taken for analysis of serum 25-hydroxyvitamin D (25[OH]D) concentration and DNA extraction. Fifteen single nucleotide polymorphisms (SNP) in 6 genes (*DBP, DHCR7, CYP2R1, CYP27B1, CYP24A1, VDR*) previously reported to associate with circulating 25(OH)D concentration were typed using Taqman allelic discrimination assays. Linear regression was used to identify environmental and genetic factors independently associated with serum 25(OH)D concentration. Mean serum 25(OH)D concentration was 42.7 nmol/L (SD 22.0); 144/222 (64.9%) participants had serum 25(OH)D concentrations <50 nmol/L. The following factors were independently associated with lower serum 25(OH)D concentration: non-white ethnicity (−8.6 nmol/L, 95% CI −14.9 to −2.3, $P = 0.008$); lack of vitamin D supplement consumption (−17.1 nmol/L, 95% CI −23.3 to −10.9, $P < 0.001$) *vs.* taking a daily supplement; sampling in Q1/January–March (−12.2 nmol/L, 95% CI −21.5 to −2.9, $P = 0.01$), and sampling in Q4/October–December (−10.3 nmol/L, 95% CI −20.2 to −0.4, $P = 0.04$) *vs.* sampling in Q3/July–September. None of the 15 SNP investigated independently associated with serum 25(OH)D concentration after correcting for multiple comparisons. In conclusion, vitamin D deficiency was highly prevalent among the older adults in this study; non-White ethnicity, lack of vitamin D supplement consumption and sampling in winter and spring independently associated with lower vitamin D status.

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1. Introduction

The risk of vitamin D deficiency is high in older adults due to the contribution of several physiological and lifestyle changes which occur with advancing age, such as a decrease in epidermal capacity to produce pre-vitamin D [1]; an increase in the prevalence of chronic kidney disease [2] and a decline in sun-seeking behavior due to avoidance or reduced mobility [3,4]. Besides these factors relating specifically to older age, several other environmental and genetic factors can further increase the risk of vitamin D deficiency in older adult populations: women often display lower 25-hydroxyvitamin D (25[OH]D) levels than men [5], possibly due to a greater adipose tissue component that sequesters the fat-soluble 25(OH)D compound from the circulation. Adipose sequestration may also be responsible for the inverse association often seen between body mass index (BMI) and 25(OH)D concentration [6,7]. Ethnicity and skin pigmentation affect 25(OH)D concentration in a number of ways: cutaneous vitamin D production depends on ultra-violet radiation (UVR) penetration which is limited by high skin melanin density [8], and ethnic variation in vitamin D pathway genes may also play a role [9]. The level of cutaneous vitamin D synthesis may also be affected by lifestyle factors which affect UVR exposure, such as the amount of time spent outdoors, the use of tanning beds, living or holidaying in sunny locations, dress-related skin exposure, and the use of sunscreen. Finally, several single nucleotide polymorphisms (SNP) in vitamin D pathway genes have been found to associate with serum 25(OH)D concentration. The most commonly known are within the vitamin D binding gene (*DBP*); 7-dehydrocholesterol reductase gene (*DHCR7*); and two cytochrome P450 enzyme genes (*CYP2R1* and *CYP24A1*) [9,10].

Vitamin D deficiency is associated with increased susceptibility to several major causes of morbidity and mortality in older adults, including fractures [11], falls [12] and acute respiratory infections [13]. It is known to be common among populations of older adults who are unable to mobilise outdoors, such as those in care homes [3,4]. However, there is relatively little data relating to vitamin D status of older adults in the UK who have better mobility, such as those living in sheltered accommodation. We therefore conducted a study to determine the prevalence of vitamin D deficiency in a cohort of older adults living in sheltered accommodation, and to identify environmental and genetic factors associating with low serum 25(OH)D concentration in this population.

2. Methods

2.1. Participants

Older adults living in 108 sheltered accommodation, residential care homes, nursing homes, or older adults' day centers in the East London area were provided an invitation letter to participate in a clinical trial of vitamin D supplementation, conducted within their community setting [14].

2.2. Procedures

At enrolment, participants were asked to complete a lifestyle questionnaire detailing age, sex, ethnicity, self-reported skin-type (Fitzpatrick skin type chart [15]), socio-economic position (SEP), amount of time spent outdoors, history of recent sunny holidays abroad, smoking behavior and consumption of alcohol and supplemental vitamin D. Researchers recorded the extent of hair loss on the head (Norwood–Hamilton [16] scale for males; Ludwig scale [17] for females), if any, skin melanin density measured using a DSM II Colormeter (Cortex Technology, Hadsund, Denmark), height (measured using a Seca 220 Telescopic Measuring Rod [Seca, Hamburg, Germany]), and weight (measured using Marsden MMPS-250 column scales [Marsden, Rotherham, UK]). A blood sample was collected for subsequent DNA extraction and determination of serum concentrations of total 25(OH)D.

2.2.1. SNP panel selection

A literature search of the Pubmed database was performed to identify SNP which have previously been shown to associate with serum 25-hydroxyvitamin D concentration. We identified 33 SNP in 6 genes in the vitamin D pathway (Table S1). Based on linkage disequilibrium (LD) relationships between these SNP, we selected 6 tagging SNP (tSNP) [18], using a r^2 threshold of 0.8, which reduced the panel of SNP we genotyped to 15.

2.2.2. Laboratory analyses

Serum concentrations of 25(OH)D_2 and 25(OH)D_3 were determined by isotope-dilution liquid chromatography–tandem mass spectrometry [19] in the Department of Clinical Biochemistry at Homerton Hospital, and summed to give total serum 25(OH)D concentration; this laboratory participates in the DEQAS external quality assurance scheme (www.deqas.org). DNA was extracted from whole blood using a salting-out method [20] on the Biomek FX robot (Beckman Coulter), and quantified using the nanodrop spectrophotometer and normalised to 5 ng/μL. 10 ng DNA was used as template for 2 μL TaqMan assays (Applied Biosystems, Foster City, CA, USA) performed on the ABI 7900HT platform in 384-well format and analysed with Autocaller software as previously described [21].

2.2.3. Statistical analysis

Using STATA 12 we performed unpaired Student's *t*-tests and one-way ANOVA to identify environmental factors and SNP that significantly ($p < 0.05$) associated with serum 25(OH)D concentration. Univariate linear regression was then performed to identify determinants of serum 25(OH)D concentration. Significant factors identified by univariate analysis were then fitted in multivariate models with each individual factor to give adjusted regression coefficients, along with a 95% confidence interval and p value. For SNP analyses, correction for multiple comparisons was then applied using the Benjamini and Hochberg method with a false discovery rate (FDR) of 5% [22]. Serum 25(OH)D concentration (nmol/L) was normally distributed and analysed as the continuous dependent variable in regression models, while independent variables were classified as categorical variables. The study was approved by East London and The City Research Ethics Committee 1 (ref 09/H0703/76) and written informed consent was obtained from all participants before enrolment.

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Table 1
Participant characteristics.

		N = 222
Sex, *n* (%)	Female	133 (59.9)
	Male	89 (40.1)
Mean age, yrs (s.d.)		72.0 (9.2)
Mean BMI, kg/m^2 (s.d.)		29.3 (6.8)
Ethnicity, *n* (%)[a]	White	163 (74.8)
	Asian/Asian British	14 (6.4)
	Black/Black British	38 (17.4)
	Mixed	3 (1.4)
Fitzpatrick skin-type, *n* (%)[b]	1	19 (8.7)
	2	44 (20.2)
	3	72 (32.0)
	4	37 (17.0)
	5	26 (11.9)
	6	20 (9.2)
Socio-economic position, *n* (%)[c]	1	72 (33.5)
	2	28 (13.0)
	3	14 (6.5)
	4	36 (16.7)
	5	62 (28.8)
	Unclassified	3 (1.4)
Time outdoors per day, *n* (%)[d]	>2 h	82 (37.6)
	≤2 h	136 (62.4)
Vitamin D supplement[e]	Yes	55 (25.8)
	No	158 (74.2)
Quarter of blood draw, *n* (%)[f]	Q1	87 (39.4)
	Q2	59 (26.7)
	Q3	24 (10.9)
	Q4	51 (23.1)
Smoking status, *n* (%)	Non-current	187 (84.2)
	Current	35 (15.8)
Mean alcohol, units/month (s.d.)[g]		5.6 (12.7)
Recent sunny holiday, *n* (%)[h]	Yes	11 (5.0)
	No	208 (95.0)
Mean melanin skin density (s.d.)[i]		36.5 (12.8)
Hair loss, *n* (%)[j]	0	123 (55.4)
	1	39 (17.6)
	2	20 (9.0)
	3	36 (16.2)
	4	4 (1.8)
Serum 25(OH)D, nmol/L (%)	<25	55 (24.8)
	25–49.9	89 (40.1)
	50–74.9	61 (27.5)
	≥75	17 (7.6)
Mean serum 25(OH)D (s.d.)		42.7 (22.0)

[a] Ethnicity not reported in *n* = 4.
[b] Fitzpatrick skin-type not reported in *n* = 4. Classification of scores: 1 = extremely fair skin; always burn, never tan, 2 = fair skin; always burn, sometimes tan, 3 = pale skin; sometimes burn, always tan, 4 = olive skin; rarely burn, always tan, 5 = moderately pigmented brown skin; never burn, always tan, 6 = markedly pigmented black skin; never burn, always tan.
[c] SEP not reported in *n* = 7. Class definitions: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations.
[d] Time outdoors not reported in *n* = 4.
[e] Supplementary vitamin D consumption not reported in *n* = 9.
[f] Quarter of blood draw not reported in *n* = 1.
[g] 1 unit is defined as 10 mL of pure alcohol.

3. Results

3.1. Study population

The population for this cross-sectional study was formed from the 222 older adults screened between 29th March 2010 and 16th March 2012 for participation in the ViDiFlu trial [14]. All screened respondents consented to anthropometric measurements, completed the lifestyle questionnaire and donated blood samples for quantification of serum 25(OH)D concentration and for DNA storage and genotyping. Participant characteristics are presented in Table 1. Participants were aged 48–94 years at enrolment; mean age was 72.0 years (SD 9.2). Females were more strongly represented in this study population (59.9%) than males. The majority ethnic group was White (74.8%); 6.4% were Asian/Asian British; 17.4% were Black/Black British; and 1.4% had mixed ethnicity. Mean serum 25(OH)D concentration for all participants was 42.7 nmol/L (SD 22.0). 55 participants (24.8%) had serum 25(OH)D concentration <25 nmol/L; 89 (40.1%) had serum 25(OH)D concentration 25–49.9 nmol/L; 61 (27.5%) had serum 25(OH)D concentration 50–74.9 nmol/L; and 17 (7.7%) had serum 25(OH)D concentration ≥75 nmol/L.

3.2. Environmental determinants of vitamin D status

Environmental determinants of vitamin D status are presented in Table 2. Multivariate analysis identified the following factors that independently associated with serum 25(OH)D concentration: Non-white ethnicity associated with a 8.6 nmol/L lower serum 25(OH)D concentration (95% CI −14.9 to −2.3, $P = 0.008$). Lack of vitamin D supplement consumption associated with a 17.1 nmol/L lower serum 25(OH)D concentration (95% CI −23.3 to −10.9, $P < 0.001$), referent to taking a vitamin D supplement. Referent to Q3/July–September sampling: Q1/January–March sampling associated with a 12.2 nmol/L lower serum 25(OH)D concentration (95% CI −21.5 to −2.9, $P = 0.01$), and Q4/October–December sampling associated with a 10.3 nmol/L lower serum 25(OH)D concentration (95% CI −20.2 to −0.4, $P = 0.04$). Sex, age, BMI, SEP, time outdoors, skin-type, skin melanin density, smoking status, alcohol consumption, recent sunny holiday and hair loss did not independently associate with serum 25(OH)D concentration in this study population.

3.3. Genetic determinants of vitamin D status

Genetic determinants of vitamin D status are presented in Table 3. After adjusting for significant lifestyle determinants of vitamin D status (ethnicity, vitamin D supplementation and season of sampling), one SNP in *DBP* independently associated with serum 25(OH)D concentration: AA genotype for rs7041 associated with

[h] Recent sunny holiday not reported in *n* = 3. Defined as a trip to any location within a latitude 51° North/South of the equator, during the local sunny season, 2 months prior to blood draw, for a duration of ≥1 week.
[i] Skin melanin density: readings taken from inside left arm, measured in arbitrary units; range of values: 16.4–90.1.
[j] Hair loss: categories 0–4 were defined by merging Male (Norwood–Hamilton [NH]) and Female (Ludwig [LG]) scales of hair loss: 0 = No hair loss, 1 = NH categories II & III (minor recession of frontal hairline; significant frontal loss/significant frontal regression + early hair loss from crown) merged with LG category I (thinning of hair from anterior crown with minimal widening of parting), 2 = NH categories IV and V (further frontal loss and enlargement of crown; further enlargement of crown and bridge begins to separate) merged with LG category II (evident thinning of crown), 3 = NH categories VI and VII (bridge disappears leaving large bald area on front and top of scalp; only back of scalp retains significant amount of hair) merged with LG category III (crown becomes denuded with significant parting, but hairline remains), 4 = other.

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Table 2
Demographic and lifestyle determinants of serum 25-hydroxyvitamin D concentration.

		N	Mean 25(OH)D, nmol/L (s.d.)	T test/One-way ANOVA P Value	Beta coefficient (95%)	P value[k]
Sex	Female	133	46.2 (21.6)	0.002	Referent	
	Male	89	37.5 (21.6)		−6.95 (−14.2 to −0.33)	0.06
Age quartiles	1 (48.0–64.5)	55	40.5 (21.8)	0.07	Referent	
	2 (64.6–71.8)	56	40.9 (22.5)		−1.64 (−9.24 to 5.96)	0.67
	3 (71.9–77.4)	55	49.7 (20.9)		+5.80 (−1.74 to 13.34)	0.13
	4 (77.5–94.1)	56	39.8 (21.9)		−3.96 (−11.62 to 3.69)	0.31
BMI, kg/m^2	<25	64	43.4 (22.7)	0.73	Referent	
	≥25	158	42.4 (21.8)		−0.42 (−6.46 to 5.61)	0.89
Ethnicity[a]	White	163	44.5 (22.3)	0.03	Referent	
	Other[b]	55	37.4 (20.9)		−8.57 (−14.86 to −2.27)	0.008
SEP[c]	1, 2	100	45.2 (21.4)	0.18	Referent	
	3, 4, 5	112	39.8 (22.3)		−2.64 (−8.37 to 3.09)	0.36
	Unclassified	3	49.7 (30.9)		−3.90 (−27.25 to 19.46)	0.74
Time outdoors, hrs/day[d]	>2	82	44.2 (22.9)	0.42	Referent	
	≤2	136	41.8 (21.7)		−1.42 (−7.02 to 4.18)	0.62
Vitamin D supplement[e]	Yes	55	56.5 (20.3)	<0.001	Referent	
	No	158	38.6 (20.3)		−17.09 (−23.28 to −10.91)	<0.001
Quarter of blood draw[f]	Q1	87	37.1 (19.6)	<0.001	−12.20 (−21.50 to −2.89)	0.01
	Q2	59	48.7 (23.6)		−2.03 (−11.65 to 7.60)	0.68
	Q3	24	54.4 (21.7)		Referent	
	Q4	51	39.3 (20.5)		−10.32 (−20.24 to −0.40)	0.04
Fitzpatrick skin-type[g]	1,2	63	42.5 (22.5)	0.19	−4.30 (−10.70 to −2.11)	0.19
	3,4	109	44.7 (22.2)		Referent	
	5,6	46	38.1 (21.0)		−0.22 (−12.30 to 11.85)	0.97
Skin melanin density quartiles[h]	1	55	39.4 (22.7)	0.17	Referent	
	2	65	45.3 (22.0)		+3.72 (−3.78 to 11.22)	0.33
	3	48	46.6 (20.9)		+5.84 (−2.33 to 14.01)	0.16
	4	54	39.5 (21.7)		+6.39 (−4.80 to 17.58)	0.26
Smoking status	Non-current	187	43.6 (22.5)	0.21	Referent	
	Current	35	38.1 (18.8)		−5.14 (−12.93 to 2.65)	0.20
Alcohol consumption, units/wk	0	87	43.8 (21.5)	0.56	Referent	
	1–20	121	41.5 (22.2)		−1.31 (−7.25 to 4.64)	0.67
	>20	14	46.5 (23.9)		+5.42 (−6.68 to 17.53)	0.38
Recent sunny holiday[i]	Yes	11	46.8 (18.8)	0.52	Referent	
	No	208	42.5 (22.2)		−4.00 (−16.15 to 8.16)	0.52
Hair loss[j]	0	123	46.1 (21.2)	0.03	Referent	
	1	39	40.3 (25.2)		+0.14 (−9.12 to 9.40)	0.98
	2	20	38.0 (17.9)		−3.51 (−14.90 to 7.89)	0.55
	3	36	34.9 (19.7)		−2.93 (−13.74 to 7.88)	0.59
	4	4	55.5 (32.0)		+14.23 (−8.82 to 37.28)	0.23

Abbreviations: s.d.: standard deviation, CI: confidence interval.
[a] Ethnicity not reported in $n = 4$.
[b] Other ethnicities: $n = 14$ Asian/Asian British, $n = 38$ Black/Black British, $n = 3$ mixed ethnicity.
[c] SEP not reported in $n = 7$. Class definitions: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations, 6 = student, 7 = never/long-term (>5 yrs) unemployed.
[d] Time outdoors not reported in $n = 4$.
[e] Supplementary vitamin D consumption not reported in $n = 9$.
[f] Quarter of blood draw not reported in $n = 1$.
[g] Fitzpatrick skin-type not reported in $n = 4$. Classification of scores: 1 = extremely fair skin; always burn, never tan, 2 = fair skin; always burn, sometimes tan, 3 = pale skin; sometimes burn, always tan, 4 = olive skin; rarely burn, always tan, 5 = moderately pigmented brown skin; never burn, always tan, 6 = markedly pigmented black skin; never burn, always tan.
[h] Skin melanin density: readings taken from inside left arm, measured in arbitrary units; range of values: 16.4–90.1.
[i] Recent sunny holiday not reported in $n = 3$. Defined as a trip to any location within a latitude 51° North/South of the equator, during the local sunny season, 2 months prior to blood draw, for a duration of ≥1 week.
[j] Hair loss: categories 0–4 were defined by merging Male (Norwood–Hamilton [NH]) and Female (Ludwig [LG]) scales of hair loss: 0 = No hair loss, 1 = NH categories II and III (minor recession of frontal hairline; significant frontal loss/significant frontal regression + early hair loss from crown) merged with LG category I (thinning of hair from anterior crown with minimal widening of parting), 2 = NH categories IV and V (further frontal loss and enlargement of crown; further enlargement of crown and bridge begins to separate) merged with LG category II (evident thinning of crown), 3 = NH categories VI and VII (bridge disappears leaving large bald area on front and top of scalp; only back of scalp retains significant amount of hair) merged with LG category III (crown becomes denuded with significant parting, but hairline remains), 4 = other.
[k] Adjusted for sex, ethnicity, vitamin D supplement consumption, quarter of blood draw, and hair loss.

10.2 nmol/L lower level (95% CI −18.7 to −1.7, $P = 0.02$), referent to CC genotype. After correcting for multiple comparison testing (Bejamini and Hochberg; false discovery rate = 0.05) this association did not remain significant.

4. Discussion

To our knowledge this is the first study to comprehensively investigate the environmental and genetic determinants of vitamin D status in a UK cohort of older adults residing in sheltered accommodation (community housing). The majority of participants (64.9%) were vitamin D deficient at the 50 nmol/L 25(OH)D threshold; the mean serum 25(OH)D concentration for all participants was 42.7 nmol/L. These levels are comparable to those reported in a recent UK national survey for over 65 year olds living in private households [23], and higher than those reported among older adults in care home settings who are unable to mobilise out of doors [4,24].

Three environmental factors independently associated with lower vitamin D status: non-white ethnicity, lack of vitamin D supplement consumption, and sampling in Winter or Spring. Non-White ethnicity (25% of our cohort) associated with a 8.6 nmol/L

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Table 3
Genetic determinants of serum 25-hydroxyvitamin D concentration.

Gene	SNP	Genotype	*N*	Mean 25(OH)D, nmol/L (s.d.)	Beta coefficient (95% CI)	*P* value[a]
CYP24A1	rs6013897	TT	128	44.6 (23.3)	Referent	
		AT	75	40.7 (19.7)	−0.20 (−5.87 to 6.27)	0.95
		AA	11	37.5 (23.5)	−5.87 (−18.13 to 7.05)	0.39
	rs2248137	CC	72	44.2 (20.3)	Referent	
		CG	94	42.3 (24.1)	−1.03 (−7.46 to 5.41)	0.76
		GG	51	40.5 (20.3)	−2.90 (−10.61 to 4.80)	0.46
DBP	rs16846876	AA	118	43.1 (22.6)	Referent	
		AT	88	42.9 (21.7)	−3.01 (−8.79 to 2.77)	0.31
		TT	15	40.9 (19.3)	−3.75 (−15.40 to 7.91)	0.53
	rs7041	CC	58	48.4 (25.4)	Referent	
		AC	103	41.7 (21.2)	−4.84 (−11.55 to 1.88)	0.16
		AA	53	37.8 (19.0)	−10.17 (−18.69 to −1.65)	0.02
	rs12512631	TT	94	43.0 (21.8)	Referent	
		CT	101	41.7 (22.6)	−1.05 (−6.91 to 4.82)	0.73
		CC	25	45.5 (21.8)	+1.94 (−7.60 to 11.48)	0.69
	rs4588	GG	131	44.2 (22.5)	Referent	
		GT	81	41.0 (21.5)	−4.23 (−9.97 to 1.51)	0.15
		TT	9	37.1 (19.4)	−9.67 (−24.29 to 4.95)	0.19
	rs2070741	TT	181	43.3 (22.3)	Referent	
		TG	29	37.3 (20.2)	−6.21 (−14.34 to 1.93)	0.13
		GG	2	44.0 (28.3)	−6.01 (−34.40 to 22.38)	0.68
	rs2298849	AA	130	42.6 (21.9)	Referent	
		AG	69	44.2 (22.0)	+0.27 (−5.79 to 6.32)	0.93
		GG	18	38.1 (24.5)	−4.28 (−14.74 to 6.18)	0.42
CYP27B1	rs4646536	AA	103	44.9 (22.8)	Referent	
		AG	87	41.8 (21.1)	−3.09 (−8.98 to 2.80)	0.30
		GG	27	39.3 (22.6)	−5.10 (−13.63 to 3.44)	0.24
CYP2R1	rs10500804	TT	97	44.3 (22.3)	Referent	
		GT	96	40.5 (21.8)	−4.26 (−10.26 to 1.74)	0.16
		GG	27	45.6 (22.3)	−3.48 (−12.72 to 5.76)	0.46
	rs2060793	GG	67	41.5 (23.3)	Referent	
		AG	114	41.9 (21.4)	+1.62 (−4.73 to 7.97)	0.62
		AA	38	47.9 (21.8)	+6.35 (−1.99 to 14.69)	0.14
	rs10766197	GG	85	41.8 (21.5)	Referent	
		AG	94	41.9 (22.8)	+0.05 (−6.35 to 6.45)	0.99
		AA	27	47.9 (23.1)	+1.37 (−8.23 to 10.97)	0.78
DHCR7	rs12785878	TT	106	45.0 (21.9)	Referent	
		GT	79	42.4 (22.7)	−2.32 (−8.77 to 4.12)	0.33
		GG	36	36.8 (20.1)	−3.04 (−12.40 to 6.31)	0.52
	rs3829251	GG	155	42.7 (21.7)	Referent	
		AG	49	41.3 (23.0)	−0.88 (−7.62 to 5.85)	0.80
		AA	8	46.0 (25.9)	+4.13 (−10.43 to 18.68)	0.58
VDR	rs10783219	AA	97	40.2 (21.5)	Referent	
		AT	90	44.1 (21.6)	+3.64 (−2.61 to 9.88)	0.25
		TT	27	45.3 (26.2)	+4.95 (−4.23 to 14.14)	0.29

Abbreviations: DBP: vitamin D binding protein, CYP2R1: cytochrome P450-2R1, CYP24A1: cytochrome P450-24A1, CYP27B1: cytochrome P450-27B1, DHCR7: 7-dehydrocholesterol reductase, VDR: vitamin D receptor, SNP: single nucleotide polymorphism, s.d.: standard deviation, CI: confidence interval.
[a] Adjusted for ethnicity, quarter of blood draw and vitamin D supplement consumption.

lower vitamin D status. Of our non-White participants, the largest groups were Black/Black British (38/55 non-White participants), and Asian/Asian British (14/55 non-White participants). Our findings agree with previous reports of vitamin D status in elderly participants: an observational study conducted in Baltimore, U.S. found lower serum 25(OH)D concentrations in African American participants aged ≥65 years, compared to White participants of a similar age [25], and one study conducted in Birmingham, UK reported a higher prevalence of vitamin D deficiency (57%) in elderly Asian participants as compared to elderly White participants (11%) [26]. Consumption of daily vitamin D supplements associated with better vitamin D status: those taking a daily supplement had a 17.1 nmol/L higher 25(OH)D level, compared to those not taking a supplement. A cross-sectional study of elderly Dutch participants reported a similar association: from combined dietary sources of vitamin D they calculated that consumption of just 6.4 μg/day (256 IU) equated to a 16.8 nmol/L higher serum 25(OH)D concentration [27]. Our analysis also highlighted a statistically significant effect of season on serum 25(OH)D concentrations. This finding is consistent with reports from two cross-sectional studies in UK adults [28,29] where vitamin D status was reported to peak in September and to trough from January to March.

Our study has several strengths. We investigated a wide range of potential environmental and genetic determinants of vitamin D status in study participants that included objective measurement of skin pigmentation using a colorimeter. Two further strengths were the measurement of serum 25(OH)D concentration by LC–MS/MS in a laboratory participating in the DEQAS scheme, and the fact that the study was conducted over a 1 year period to allow observation of serum 25(OH)D concentrations across all seasons.

Our study also has some limitations. One limitation was the relatively small sample size we had to investigate genetic determinants of vitamin D status—whilst the direction of association between SNP genotype and 25(OH)D concentrations in our study population were in agreement with those reported in previous studies, the paucity of statistically significant associations we identified may have arisen due to a lack of statistical power for detection. One further limitation was the estimate of UVR exposure data by self-reported outdoor activity rather than by direct measurement of UVR exposure.

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In conclusion, we report that vitamin D deficiency was highly prevalent in a population of UK older adults living in sheltered accommodation, despite their ability to mobilise independently outdoors. Non-white ethnicity, lack of vitamin D supplement consumption and sampling in Winter and Spring independently associated with decreased serum 25(OH)D concentrations in this population, but genetic variation in the vitamin D pathway did not. The clinical implication of our findings is that consumption of vitamin D supplements is protective against vitamin D deficiency in this population, and vigorous efforts to improve uptake of such supplements should be made.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsbmb.2016.01.005.

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