From INSTITUTE OF ENVIRONMENTAL MEDICINE
Karolinska Institutet, Stockholm, Sweden

OCCUPATIONAL FACTORS AND RISK OF RHEUMATOID ARTHRITIS: STUDIES ON SILICA, PHYSICAL WORKLOAD AND COLD WORK ENVIRONMENT

Pingling Zeng
曾萍铃

Karolinska Institutet

Stockholm 2017

Published by Karolinska Institutet.
Printed by E-Print AB 2017

ISBN 978-91-7676-845-7

Occupational Factors and Risk of Rheumatoid Arthritis: Studies on Silica, Physical Workload and Cold Work Environment

THESIS FOR DOCTORAL DEGREE (Ph.D.)

This thesis will be defended in public at the Rockefeller lecture hall, Nobels väg 11, Karolinska Institutet, Solna Campus

Thursday the 14th of December 2017, at 13:00

By

Pingling Zeng

Principal Supervisor:
Professor Lars Alfredsson
Karolinska Institutet
Institute of Environmental Medicine
Unit of Cardiovascular Epidemiology

Co-supervisors:
Assistant Professor Camilla Bengtsson
Karolinska Institutet
Institute of Environmental Medicine
Unit of Cardiovascular Epidemiology

Professor Lars Klareskog
Karolinska Institutet
Department of Medicine Solna
Unit of Rheumatology

Mentor:
Professor Alasdair Gibb
University College London
Department of Neuroscience Physiology and Pharmacology

Opponent:
Professor Jonas Björk
Lund University
Division of Occupational and Environmental Medicine

Examination Board:
Professor Hui-Xin Wang
Stockholm University
Stress Research Institute

Professor Maria Albin
Karolinska Institutet
Institute of Environmental Medicine
Unit of Occupational Medicine

Dr. Ulla Lindqvist
Uppsala University
Department of Medical Sciences, Rheumatology

ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease that could lead to joint destruction and disabilities. Around 30-70 million people worldwide are affected by RA. Both genetic and environmental factors are involved in the etiology of RA. However, researches aiming at understanding the environmental factors leading to RA are relatively few.

Aims: The main aim of this thesis is to investigate occupational risk factors for RA. The sub-aims are: 1.) To investigate the interaction between the dose of cigarette smoking and silica exposure regarding risk of anti-citrullinated protein antibody (ACPA) positive RA among male subjects. 2.) To investigate the association between different types of physical workload and risk of RA. 3.) To investigate the association between different types of physical workload and development of antibodies against collagen type II (anti-CII) in RA. 4.) To investigate the association between working in cold environment and risk of RA.

Materials and Methods: This thesis was based on the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) Study, which is a population-based case-control study involving more than 3000 incident RA cases and more than 5000 controls recruited between 1996 and 2014. Information on exposures (i.e. cigarette smoking, silica, physical workload and cold work environment) was obtained through self-reported questionnaire. RA cases were ascertained based on the ACR 1987 or 2010 criteria. The associations between exposures and outcome (risk of developing RA) were estimated using logistic regression by calculating the odds ratios (OR) and 95% confidence intervals (CI). Presence of additive interaction between risk factors was evaluated by calculating the attributable proportion due to interaction (AP).

Results: As the dose of smoking increased, the magnitude of interaction between silica and smoking also increased, with the highest AP value (AP=0.7; 95%CI: 0.4-0.9) observed at 28 pack-years of smoking. The interaction between silica and smoking among those who quitted smoking for ⩽10 years was estimated to have an AP of 0.5 (95%CI: 0.1-0.9). The associations between different types of physical workload and risk of developing RA ranged from 1.3(95%CI: 1.1-1.4) to 1.8(95%CI: 1.6-2.0). The ORs between physical workloads and risk of developing anti-CII positive RA was comparable with the corresponding ORs observed between physical workloads and risk of developing anti-CII negative RA. Those who reported they had worked in cold environment had a 50% higher risk of developing RA than those who did not (OR=1.5 (95%CI:1.4-1.7)).

Conclusions: The interaction between smoking and silica depends on the cumulative dose of smoking and the effect of interaction could remain even after nearly 10 years of smoking cessation. Both exposure to physical workload and exposure to cold work environment were found to be associated with an increased risk of developing RA. These factors are new potential risk factors for RA and need confirmation. The findings may provide new clues for unraveling the etiology of RA and contribute to the endeavor of making RA a preventable and curable disease.

LIST OF SCIENTIFIC PAPERS

I. **Zeng P**, Chen Z, Klareskog L, Alfredsson L, Bengtsson C, Jiang X. *Amount of smoking, duration of smoking cessation and their interaction with silica exposure in the risk of rheumatoid arthritis among males: results from the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study* Annals of the Rheumatic Diseases Published Online First: 15 September 2017. doi: 10.1136/annrheumdis-2017-212145.

II. **Zeng P**, Klareskog L, Alfredsson L, Bengtsson C. *Physical workload is associated with increased risk of rheumatoid arthritis: results from a Swedish population-based case–control study*. RMD Open 2017;3:e000324.

III. **Zeng P**, Alfredsson L, Klareskog L, Mullazehi M, Saevarsdottir S, Bengtsson C, Rönnelid J. *Occupational physical workload and development of anti-collagen type II antibodies in rheumatoid arthritis*. (manuscript)

IV. **Zeng P**, Bengtsson C, Klareskog L, Alfredsson L. *Working in cold environment and risk of developing rheumatoid arthritis: results from the Swedish EIRA case–control study*. RMD Open 2017;3:e000488. doi: 10.1136/rmdopen-2017-000488.

CONTENTS

LIST OF ABBREVIATIONS

ACPA	Anti-Citrullinated Protein Antibody
ACR	American College of Rheumatology
Anti-CII	Antibodies against Collagen Type II
EIRA	Epidemiological Investigation of Rheumatoid Arthritis
EULAR	European League Against Rheumatism
HLA	Human Leukocyte Antigen
HSP	Heat Shock Protein
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
SE	Shared Epitope

1 INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by inflamed synovial tissue which could lead to joint destruction and disabilities. It affects 0.5%-1% of the world population. There is no available cause-directed curative therapies for RA, and this would not be possible without better understanding of the causes of RA. Aside from genetic predisposition, environmental factors play a substantial role in RA etiology. There are vast amount of researches on RA focusing on the cellular or molecular level to understand the disease pathogenesis. In contrast, relatively few researches on RA are focused on modifiable environmental factors. Providing new knowledge on environmental risk factors would address patients' need and desire to understand why they develop the disease and how to prevent their family members from suffering from the same disease. Thus, the overall aim of this thesis is to increase our knowledge on environmental risk factors of RA.

This thesis is made possible through the use of the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study, which is a population based case-control study. The EIRA study currently possesses one of the world's largest and most complete data that contain both environmental and genetic information about incident RA cases and their corresponding controls. This thesis analyzed data from more than 3000 incident RA cases and more than 5000 controls recruited between 1996 and 2014.

By using the data from EIRA study, our research group has previously found an interaction between smoking and silica with regards to the risk of anti-citrullinated protein antibodies (ACPA)-positive RA; however the dose of smoking needed to elicit such interaction effect was unknown. Therefore, **study I** aimed at investigating the interaction between silica exposure and dose of smoking as well as between silica exposure and duration of smoking cessation, with regard to the risk of developing ACPA-positive RA.

Physical workload is a potential risk factor that needs to be consider for all types of joint disease including RA, since the joints are directly involved in performing physical workload. The aims of **study II** and **study III** were to investigate the association between physical workload and risk of RA, as well as the relation between physical workload and 1.) RA related gene (HLA-DRB1 shared epitope); 2.) development of two auto-antibodies (ACPA and antibodies against type II collagen) in RA.

Another potential risk factor for RA is exposure to cold environment. Coldness has been suspected to be associated with arthritis for a long time in human history. Although there are many studies that investigated meteorological factors and RA signs and symptoms, studies that investigate the association between working in cold environment and risk of developing RA in healthy individuals have not been found in the literature. Therefore, **study IV** aimed at shedding some light on this missing knowledge.

2 BACKGROUND

2.1 CLINICAL FEATURES AND CLASSIFICATION OF RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease characterized by inflamed synovial tissue which may eventually lead to cartilage and joint destruction.[1] In addition to the inflamed joints, it may also exhibit various extra-articular manifestations, such as rheumatoid nodules, vasculitis and interstitial lung disease.[2] RA is associated with increased mortality and several systemic comorbidities including cardiovascular disease, interstitial lung disease and cerebrovascular events.[3,4]

Clinical features of RA include swollen and tender joints, morning joint stiffness, elevated level of C-reactive protein or erythrocyte sedimentation rate. However, these features are not specific for rheumatoid arthritis, since similar features are also seen in other types of arthritis such as psoriatic arthritis and some inflammatory connective tissue diseases.[5] Although there are no specific diagnostic criteria for rheumatoid arthritis, classification criteria were developed to facilitate the identification of a homogenous population for research purposes and for supporting diagnosis.

The 1987 American College of Rheumatology (ACR) criteria (**table 1**) was created based on studies from subjects with well-established RA.[6] This method has a high sensitivity and specificity for classifying established RA, but has a low sensitivity and specificity in classifying early RA.[7] Clinical studies have shown that identifying and treating RA patients at an early stage can achieve better treatment outcome by slowing or preventing bone/cartilage damage, decreasing disability and increasing the rate of disease remission.[8,9] To overcome the limitation of the 1987 ACR criteria, the 2010 ACR/EULAR criteria (**table 1**) was developed using cohorts of subjects with early RA.[10] It includes additional serological markers (rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA)), long symptom duration and laboratory markers of systemic inflammation.

Table 1: Classification Criteria for Rheumatoid Arthritis (RA)		
	1987 ACR Classification Criteria	**2010 ACR/EULAR Classification Criteria**
criteria	1. Morning stiffness (at least one hour) 2. Arthritis in three or more join areas 3. Arthritis of hand joints (≥1 swollen joints) 4. Symmetric arthritis 5. Rheumatoid nodules 6. Serum RF 7. Radiographic changes (erosions) on X-rays of hands	1. Joint involvement (0-5) • medium-large joint [a] (0) • 2-10 medium-large joints (1) • 1-3(small joints[b] (large joint not counted) (2) • 4-10 small joints (large joint not counted) (3) • >10 joints (at least one small joints) (5) 2. Serology (0-3) • negative RF and negative ACPA[c] (0) • low positive RA or low positive ACPA[d] (2) • high positive RA or high positive ACPA[e] (3) 3. Acute phase reactants • normal CRP and normal ESR[f] (0) • abnormal CRP or abnormal ESR (1) 4. Duration of symptoms (0-1) • <6 weeks (0) • ≥6 weeks (1)
applicable for	patients with established RA	patients with early or established RA
positive in cases	Four of the seven criteria must be present. Criteria 1-4 must have been present for at least six weeks.	Scoring ≥6 points. In the presence of typical erosions seen in light of an inflammatory disorder or long-standing disease previously satisfying the classification criteria, no other points need to be obtained for the classification of RA.

Scoring points are shown in parentheses.

[a] Large joints refer to shoulders, elbows, hips, knees, and ankles.

[b]Small joints refer to the metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists.

[c]Negative refers to IU values that are less than or equal to the upper limit of normal (ULN) for the
laboratory and assay.

[d]low-positive refers to IU values that are higher than the ULN but ≤3 times the ULN for the laboratory and assay.

[e]high-positive refers to IU values that are >3 times the ULN for the laboratory and assay. Where rheumatoid factor (RF) information is only available as positive or negative, a positive result should be scored as low-positive for RF.

[f]Normal/abnormal is determined by local laboratory standards.

RF=rheumatoid factor; ACPA,=anti-citrullinated protein antibody; CRP= C-reactive protein; ESR,=erythrocyte sedimentation rate

2.2 TREATMENT STRATEGY FOR RHEUMATOID ARTHRITIS

Therapeutic approaches for RA have evolved from non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids to disease-modifying anti-rheumatic drugs (DMARDs). DMARDS target inflammation, reduce pain and swelling, improve physical function and modify or limit progressive joint damage.[5] According to the EULAR recommendations, conventional DMARD, preferably methotrexate, should be the choice for initial treatment.[11] If a patient failed to respond to initial conventional DMARDS, then he/she may be switched to biologic DMARDS or Janus kinase inhibitors and may also be treated with a combination of the numerous available drugs.[5]

Although advancements in therapeutic strategies over the past years have led to better disease activity control for RA patients, substantial portion of patients fail to respond to initial methotrexate treatment or first anti-TNF treatment,[12] and approximately 20% of patients continue to suffer from pain, joint damage and disability.[13] Failure of initial treatments would lead to a series of experimentation with other available therapeutics, and consequently would prolong and increase patients' disease burden. Timely identification and application of effective treatment would improve the efficacy of treatment management and prevent manifestation of debilitating RA. Better understanding of the causes or pathogenesis of RA is needed in order to provide cause-directed curative therapies and ultimately prevent RA.

2.3 PATHOGENESIS OF RHEUMATOID ARTHRITIS

2.3.1 Auto-Antibodies

RA is a disease caused by immune dysregulation. One of the perpetrators of immune dysregulation is the development of excessive auto-antibodies. Examples of auto-antibodies found in RA include rheumatoid factor (RF), anti-citrullinated protein antibody (ACPA) and anti-collagen type II (anti-CII) antibodies.

Rheumatoid factor (RF) are antibodies that bind to the Fc portion of IgG antibody. RF possesses diagnostic and predictive value for RA. It can be detected in around two thirds of RA patients, but can also be found in patients with other diseases such as Sjögren's syndrome, hepatitis and tuberculosis, and in a small proportion of healthy individuals.[14] Despite its low specificity, the presence of RF is associated with disease severity and it has been used as a biomarker for diagnosis and classification of RA for more than three decades.[15]

The diagnostic value of antibodies to citrullinated antigens (ACPAs) was discovered 20 years ago.[16,17] The presence of these antibodies is restricted to a subset of RA patients characterized by its association with certain HLA-DR alleles and cigarette smoking.[18] Unlike RF, ACPA has a high specificity (around 95%) for RA.[19] Individuals that are positive for RF

and/or ACPA are often called “seropositive” and constitute approximately 2/3 of the population with newly onset RA.

Approximately 15 years ago, Rantapaa et al. and Nielen et al. discovered that antibodies against citrullinated peptide can be detected in blood samples of individuals several years before they eventually developed RA.[20,21] The titer of ACPA increases when individuals get close to the onset of RA, whereas very few patients develop ACPAs after onset of RA.[20,21] ACPA positive patients have different clinical phenotypes and outcomes when compared to ACPA-negative RA. ACPA positive RA patients tend to have more aggressive disease phenotype, more bone erosion and worse disease outcome.[22] ACPA-positive RA and ACPA-negative RA also represent two distinct disease subsets in which the underlying disease pathogeneses have partially different molecular mechanisms.[23] Therefore, it is important to analyze these two subsets separately when studying RA etiology.

Anti-CII antibodies are antibodies that recognize type II collagen of the articular cartilage and plays a role in cartilage destruction. One of the functions of articular cartilage is to facilitate mechanical stress loaded on joints.[24] Notably arthritis induced by immunization with collagen type II is one of the main arthritis models in mice and rats.[25] However, immunity to native and unmodified collagen II is rare in humans. Anti-CII is present in 6-9% of RA patient at diagnosis and the titer of anti-CII decreased gradually after diagnosis.[26] In contrast to ACPA, the presence of anti-CII predicts lower degree of inflammation during the disease course and better disease outcome.[26]

The presence of these different auto-antibodies in RA suggest that loss of immune tolerance is a key event in the pathogenesis of seropositive RA. The factors that contribute to the break of immune tolerance remains to be elucidated. Identification of new environmental factors would warrant a better understanding of the event cascades leading to the development of these pathogenic auto-antibodies.

2.3.2 Genetic Factors

RA is caused by the interplay between genetic and environmental factors. The heritability of RA is estimated to be around 37-65%,[27] and is found to be higher among for sero-positive disease (50%) subset than among sero-negative (20%) subset.[28] The relatively low heritability of RA especially among the sero-negative subgroup suggests that environmental factors play a sizeable role in the etiology of RA.

According to genome wide association studies, the HLA-DRB1 locus is the dominant genetic risk factors for RA.[29] The HLA-DRB1 alleles encode for common amino acid motifs including the shared epitope (SE), which is located in the β-chain of the MHC molecule involved in antigen presentation and selection of T-cell repertoire. The role of these genes indicate that immune mechanisms, particularly peptide binding, are strongly involved in the pathogenesis of seropositive RA. Notably, the disease causing effects of these genes are triggered by environmental factors. Smoking have been shown to increase the risk of developing ACPA-positive RA especially among individuals with HLA-DRB1 risk alleles.[30] The presence of gene-environment interaction between the HLA-DRB1 alleles and smoking highlights the importance of considering gene-environment interaction when investigating the etiology of RA.

2.3.3 Pre-RA Stages

Systemic autoimmunity in RA may begin many years before symptom appearance and diagnosis. Over the years, many researches have delved into research questions related to events that occur before clinical symptoms of RA become evident. In 2011, the EULAR Study Group for Risk Factors for Rheumatoid Arthritis developed a nomenclature to describe various phases or events that could occur before diagnosis of RA.

There are five identified phases that an individual at risk of RA might undergo (**figure1**).[31] The development of RA can start as early as having the related genes and being exposed to certain environmental risk factors (phase A and B). Phase C pertains to individuals with systemic autoimmunity associated with RA. Phase D denotes individuals showing symptoms without clinical arthritis. Phase E refers to individuals with unclassified arthritis. Some individuals may develop from phase A or B to phase C (having systemic autoimmunity), while other individuals may skip phase C and proceed directly to phase D or phase E or phase F.

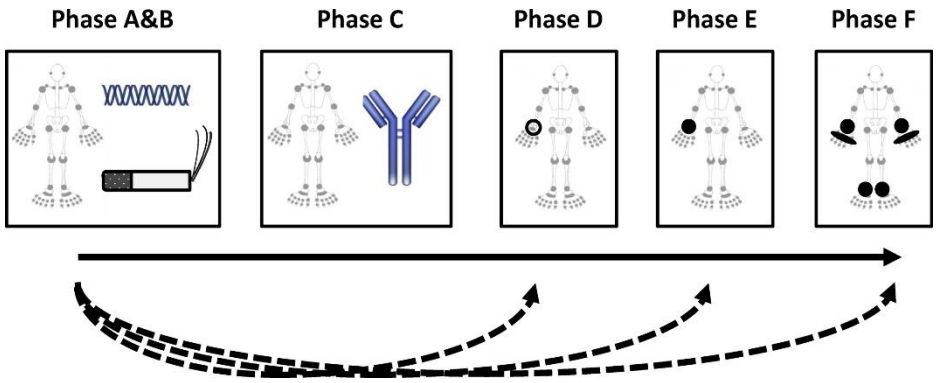

Figure 1. The possible 5 phases of RA development. The open circles represent painful joints but not swollen. The solid circles/shapes represent joints with clinically apparent soft tissue swelling. (Adapted from Raza et al. 2014).

Several studies have recruited cohorts who are in the at-risk phase of RA and performed observational studies. Some studies looked into potential pre-RA therapeutic intervention that could delay or halt the development of RA among the at-risk population.[32-35] Other studies aimed at developing algorithmic model based on the known risk factors and biomarkers to predict the risk of developing RA.[36]

Given the above-mentioned knowledge, modification of lifestyle or environmental risk factor is thus pivotal in preventing RA from a very early stage. For example, smoking cessation could have substantial RA preventive effect especially for the individuals with genetic predisposition. Since better preventive strategies are needed, it is necessary to identify and validate more environmental risk factors. When searching for potential lifestyle and environmental risk factors, taking patients' concern and perspective into considerations could be valuable. Clinicians often receive questions from patients on whether musculoskeletal workload or exposure to cold environment are contributing factors to their disease. There is, however, a lack of scientific studies related to these two commonly asked questions. Therefore, one of the aims of this thesis is to provide clues to these two commonly asked questions by conducting epidemiologic studies to investigate whether occupation physical workload and cold work environment are associated with the development of RA.

3 EPIDEMIOLOGY OF RHEUMATOID ARTHRITIS

RA affects between 0.5% to 1% of the world population.[37] Women are two to three times more likely to have RA than men.[38] Higher prevalence was observed in northern hemisphere (i.e. Northern Europe and North America) than southern hemisphere.[39] Notably, Native American groups including the Pima, Yakima and Chippewa have very high prevalence.[40] In Sweden, the incidence is 41 per 100,000, with 56/100,000 for women and

25/100,000 for men.[41] The incidence rate increases with age and highest incidence is observed in the 70-79 years age group.

Rheumatoid arthritis incurs substantial socioeconomic and individual burden. This burden may comprise of hospital care, drug cost, loss of work capacity due to physical function disability and declined quality of life. In Sweden, the annual societal cost was estimated to be around €23 000 per prevalent RA case among the non-pension age population, and around €6400 per prevalent RA case among the pension age population.[42] These costs correspond to 2-3 times higher cost as compared with the general population with no RA.[42]

3.1 ENVIRONMENTAL RISK FACTORS

3.1.1 Cigarette Smoking

Among all the environmental risk factors studied for RA, cigarette smoking is the most well-studied factor. Smokers have approximately 2-fold higher risk of RA than non-smokers.[43-46] Furthermore, a dose-response effect has been observed in several studies. [43,44,46] The association is subsequently found to be mainly confined to the anti-citrullinated protein/peptide antibody (ACPA)-positive RA;[44] and the risk appears to be not due to nicotine but due to some inhaled particles.[47] A few studies found a reduction, but not elimination of RA risk after 10-20 years of smoking cessation.[43,46,48,49]

3.1.2 Silica

Exposure to silica is a well-defined airborne hazard present in mining, quarrying, drilling and some electronic industries. Silica dust has been postulated as an etiologic agent for several systemic autoimmune diseases including RA.[50-52] Our research group has previously observed an association between silica exposure and increased risk of sero-positive RA (odds ratio (OR) 1.6 for ACPA-positive RA and 1.9 for rheumatoid factor positive RA).[53,54] We also observed an interaction effect between silica exposure and current smoking regarding ACPA-positive RA (attributable proportion due to interaction was 60%) and the OR was 7.4 among those who were both silica exposed and current smokers compared with unexposed.[54] However, the amount of smoking that could elicit this interaction effect is unclear.

3.1.3 Other Environmental Risk Factors

Several environmental risk factors for RA have been studied as shown in **table 2**. Infectious agents have been suspected to be associated with RA for several decades. There are increasing number of studies focusing on the association between Porphyromonas gingivalis and risk of RA. Porphyromonas gingivalis is considered as one of the main

organisms causing periodontal disease. It could citrullate peptides through peptidylarginine deiminase enzymes; thus, could potentially trigger the production of ACPA. However, this hypothesized association between Porphyromonas gingivalis and RA has not been supported by population-based studies.[55,56] Moderate alcohol consumption and taking omega-3 fatty acid supplements or oral contraceptives were observed to lower the risk of RA.[57-59] Hormonal factors and breastfeeding have been a longstanding interest for many epidemiological investigations concerning RA risk factors,[60-62] since RA is a disease with higher prevalence in women than men. Overall, there is a paucity of information on environmental triggers of RA, and the number of scientific researches dedicated to environmental risk factors for RA is relatively few when compared to experimental researches.

Table 2. Environmental Factors Related to Rheumatoid Arthritis

Factors generally deemed as associated with RA Risk	
• smoking, silica • lower socioeconomic status • educational attainment	• high BMI • high birthweight
Factor generally deemed as associated with RA protection	
• moderate alcohol consumption	• fish/omega-3 fatty acid consumption
Inconclusive Factors	
• diet (red meat, proteins, fruit, caffeine etc.) • psychological stress • textile dust • microbes, infectious agent (Porphyromonas gingivalis, Escherichia coli and Epstein-Barr virus)	• air pollution • breastfeeding • oral contraceptives • low Vitamin D level • occupation in manufacturing sector

4 PHYSICAL WORKLOAD

Physical workload and physical activities or exercises differ in quantity, intensity and duration of mechanical stress exerted on the joint and synovia. Examples of different kinds of physical workload at work places are shown in **table 3**. Physical workload is considered as a risk factor for non-autoimmune osteoarthritis and low back pain.[63-65] Exposure to vibration has previously been studied as a potential risk factor for RA, but the study size was small.[50] Since the joints and muscles are directly affected by physical workload, it is a potential risk factor to consider for all types of joint diseases including RA. However, studies that systematically investigate the association of occupational exposure to different types of physical workload and risk of developing RA have not been found in the literature.

Table 3. Examples of Occupational Physical Workload
1. bending/turning in a repetitive manner several times per hour
2. repetitive hand or finger movements several times per minute, for example typing or sorting
3. lift or carry objects heavier than 10 kg
4. performing precision work, such as fine mechanics, clock-making or dental work, for more than a total of 2 hours per day
5. work movements where hands are placed below knee level for more than a total of 30 minutes per day, for example floor or ground work.
6. work movements where hands are placed above shoulder level for more than a total of 30 minutes per day.
7. vibration, such as sitting in a car , boat , airplane, tractor or lorry or using hand-held vibrating machines.

5 WORKING IN COLD ENVIRONMENT

Cold exposure at work may be derived from exposure to cold air, cold water or cold surfaces. Working in cold condition may have several adverse effects on human health. It may affect respiratory illnesses such as asthma or chronic obstructive pulmonary diseases,[66,67] musculoskeletal illness such as back and neck pain,[68 69] cardiovascular disease such as acute myocardial infarction ,[70,71] and dermatological illnesses such as cold urticaria and pernio (chilblains).[72,73] It may also cause various cold associated injuries or symptoms such as frostbite, shortness of breath and numbness.[74] Both cold indoor and cold outdoor work have been associated with musculoskeletal pain, aches or muscle weakness.[69,75-77]

Coldness has been suspected to be associated with arthritis for a long time in human history. Around 400 B.C. the Greek physician Hippocrates mentioned in his book “On Air,

Waters and Places" about the influence of climate and weather conditions on joints. Interestingly, the English word "rheumatism" is known or translated as "feng shi bing" (风湿病) in Chinese, which literally means "wind wet disease". According to an influential Chinese traditional medicine book "The Medical Classic of Yellow Emperor", written more than 2000 years ago, rheumatism is considered as a disease caused by wind, coldness and wetness.

Some RA patients believe that they can predict weather changes based on their symptoms. RA disease activity and its association with seasonal changes and weather variables such as low temperature, sunshine and atmospheric pressure have been studied.[78,79] RA related pain have been hypothesized to be correlated with temperature, humidity, sunshine and atmospheric pressure,[80] but a systematic review of 9 studies reported that there is no evidence of a correlation.[81] Although there are many studies that investigated meteorological factors and RA signs and symptoms, studies that investigate the association between working in cold environment and risk of developing RA in healthy individuals has not been found in the literature.

6 AIMS

The main aim of the thesis is to identify occupational risk factors for RA.

The specific aims of the thesis are:

- **Study I:**

  To investigate the interaction between silica and 1.) dose of smoking as well as 2.) duration of smoking cessation with regards to the risk of developing ACPA-positive RA among males.

- **Study II:**

  1.) To investigate the association between occupational physical workload and risk of developing RA (overall), ACPA-positive RA and ACPA-negative RA.

  2.) To investigate the interaction between physical workload and the shared epitope gene with regards to the risk of developing ACPA-positive RA.

- **Study III:**

  To investigate the association between occupational physical workload and the development of antibodies against type II collagen (anti-CII) in RA patients

- **Study IV:**

  1.) To investigate the association between working in cold environment and risk of developing RA(overall), ACPA-positive RA and ACPA-negative RA.

  2.) To investigate the interaction between working in cold environment and physical workload.

7 MATERIALS AND METHODS

This thesis is based on the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) Case-Control Study. **Study I** analyzed only male participants recruited from 1996-2014. **Study II and IV** involved both male and female EIRA participants recruited from 1996-2014. **Study III** included cases recruited from 1996 to 2006 and controls recruited from 1996-2014.

7.1 STUDY DESIGN

Although EIRA is an on-going population-based case-control study, this thesis only included data collected from 1996 to 2014. The study base is comprised of subjects aged 18 and above living in the middle or southern part of Sweden.

7.1.1 Identification of Cases and Controls

Incident RA cases were defined as those who were newly diagnosed by rheumatologists with RA based on the American College of Rheumatology (ACR) 1987 or ACR 2010 criteria. Incident cases were recruited from all hospital-based rheumatology units and almost all private rheumatology clinics in the study area. The mean time from the appearance of first disease symptom to diagnosis was 10 months. The year when the first disease symptom appeared was defined as index year.

Controls were randomly selected from the Swedish population register and matched with the cases by age, sex and residential area. From 1996-2006, each incident case was matched with one control. From 2006 onwards, two controls were matched to each incident case. If a case was later revealed to be not fulfilling the inclusion criteria, his/her corresponding controls were retained in the study and were included in the non-matched analyses.

7.1.2 Data Collection

Cases were given a questionnaire and invited to donate a blood sample when they received their diagnosis at the clinic. Questionnaires for controls were sent by post. Unclear or missing answers in the questionnaires were clarified and completed by trained staffs through phone calls or mail. In total, 3724 (94%) of the cases and 5935 (77%) of the controls completed the questionnaire. Blood samples were collected from 99% of the cases and 55% of the controls who completed the questionnaire.

7.2 EXPOSURE ASSESSMENT AND CLASSIFICATION

7.2.1 Cigarette Smoking

Current smokers were defined as individuals who reported they had regularly smoked cigarettes during the index year. Past smokers were defined as subjects who had smoked cigarettes before but not during the index year. Never smokers were defined as subjects who had never smoked cigarettes. Cumulative dose of smoking was expressed in pack-years. One pack-year is equivalent to smoking 20 cigarettes per day for 1 year. Among past smokers, duration of smoking cessation was defined as the number of years since quitting smoking. Subjects who smoked pipe or cigar were excluded.

7.2.2 Silica

Subjects who reported they were exposed to rock drilling, stone crushing and stone dust were considered as exposed to silica. Occupations that involved rock drilling, stone crushing and stone dust were found to be highly exposed to silica.[82,83]

7.2.3 Occupational Physical Workload

Information on physical workload exposure was collected by means of questionnaire shown in **table4**. The validity of the questions to assessed physical workload exposure was found to be acceptable in other studies,[84-86] and the reproducibility and validity were further improved when the exposure is dichotomized. Subjects who answered: "never or rarely" or "not at all" were considered as unexposed to the type of physical workload in question. Subjects who gave all other answers except 'not working' were defined as exposed to the particular type of physical workload in question. Those who answered not working at baseline were excluded from the baseline exposure analysis. Similarly, those who answered not working at five years before baseline were also excluded from the 5 years exposure analysis.

Table 4. Physical Workload Questionnaire

1. **Does/did your work require you to bend over or turn in a repetitive manner several times per hour?**

Currently:	Five years ago:
1. *never or rarely*	1. *never or rarely*
2. *1-3 days/month*	2. *1-3 days/month*
3. *1 day/week*	3. *1 day/week*
4. *2-4 days/week*	4. *2-4 days/week*
5. *every working day*	5. *every working day*
6. *not working*	6. *not working*

2. Does/did your work involve performing repetitive hand- or finger-movements several times per minute? (for example, typing or sorting)

Currently:	Five years ago:
1. never or rarely	*1. never or rarely*
2. 1-3 days/month	*2. 1-3 days/month*
3. 1 day/week	*3. 1 day/week*
4. 2-4 days/week	*4. 2-4 days/week*
5. every working day	*5. every working day*
6. *not working*	*6. not working*

3. Do/did you lift or carry objects heavier than 10 kg?

Currently:	Five years ago:
1. never or rarely	*1. never or rarely*
2. 1-3 days/month	*2. 1-3 days/month*
3. 1 day/week	*3. 1 day/week*
4. 2-4 days/week	*4. 2-4 days/week*
5. every working day	*5. every working day*
6. *not working*	*6. not working*

4. Does/did your work require you to perform precision work for more than a total of two hours per day? (for example, fine mechanics, clock-making or dental work)

Currently:	Five years ago:
1. never or rarely	*1. never or rarely*
2. 1-3 days/month	*2. 1-3 days/month*
3. 1 day/week	*3. 1 day/week*
4. 2-4 days/week	*4. 2-4 days/week*
5. every working day	*5. every working day*
6. *not working*	*6. not working*

5. Does/did your work involve movements where your hands are placed below knee level for more than a total of 30 minutes per day? (for example, floor or ground work)

Currently:	Five years ago:
1. never or rarely	*1. never or rarely*
2. 1-3 days/month	*2. 1-3 days/month*
3. 1 day/week	*3. 1 day/week*
4. 2-4 days/week	*4. 2-4 days/week*
5. every working day	*5. every working day*
6. *not working*	*6. not working*

6. Do/did you perform work where your hands are/were placed above shoulder level for more than a total of 30 minutes per day?

Currently:	Five years ago:
1. never or rarely	*1. never or rarely*
2. 1-3 days/month	*2. 1-3 days/month*
3. 1 day/week	*3. 1 day/week*
4. 2-4 days/week	*4. 2-4 days/week*
5. every working day	*5. every working day*
6. not working	*6. not working*

7. What proportion of your working day do/did you work on a vibrating floor or seat? (for example, in a car, boat, aeroplane, tractor, or lorry)

Currently:	Five years ago:

1. *not at all*
2. *¼ time*
3. *½ time*
4. *¾ time*
5. *full-time*
6. *not working*

1. *not at all*
2. *¼ time*
3. *½ time*
4. *¾ time*
5. *full-time*
6. *not working*

8. What proportion of your working day do/did you work using vibrating hand-held machines?(for example, power drill, sander, nail gun, chainsaw, levers, steering wheels, etc.)

Currently:

1. *not at all*
2. *¼ time*
3. *½ time*
4. *¾ time*
5. *full-time*
6. *not working*

Five years ago:

1. *not at all*
2. *¼ time*
3. *½ time*
4. *¾ time*
5. *full-time*
6. *not working*

7.2.4 Working in Cold Environment

Information on exposure to cold work environment was collected through the questions shown in **table 5**. Subjects were asked whether or not they had ever worked in the cold and whether or not they had ever worked outdoor. Those who reported they had worked in the cold but never outdoor were considered as exposed to cold indoor work environment. Those who reported they had worked in the cold and outdoor environment, and the time periods of working in the cold and working outdoor coincide, were considered as exposed to cold outdoor work environment.

When the first year of exposure to cold work environment came after the year when the first disease symptom appeared (i.e. index year), the exposure status was regarded as unexposed. Subjects whose first year of exposure to cold work environment was the same as the index year were excluded (21 cases (0.6%) and 10 controls (0.2%)). Current exposed were defined as those who had been working in the cold environment before index and were still working in the cold environment during index year. Past exposed were defined as those who had been working in the cold environment before index year but not at index year.

Table 5. Questionnaire for Working in Cold Environment

			Time Period 1		**Time Period 2**	
	No	Yes	from-to what year	number of hours/week	from-to what year	number of hours/week
work in the cold						
outdoor work						

7.3 ANTIBODY ASSAYS AND GENOTYPING

The level of ACPA in the serum samples was measured using enzyme-linked immunosorbent anti-CCP2 assay (Immunoscan RA Mark2, Euro-Diagnostica, Malmö, Sweden). The cut-off value for positive result was ≥25 units/mL.

The level of anti-CII in the serum samples was measured using ELISA. The cut-off value for positive result was 29AU/mL. ELISA readings that showed non-specific binding were treated separately.[26]

HLA-DRB1 genotyping was performed using sequence-specific primer-PCR (DR low-resolution kit; Olerup SSP, Saltsjöbaden, Sweden). HLA-DRB1*01, HLA-DRB1*01*04, HLA-DRB1*01*10 were defined as shared epitope.[30]

7.4 POTENTIAL CONFOUNDERS

Potential confounders included in the analyses were: educational level (university degree, yes or no) body mass index (BMI, <25 kg/m^2 or ≥ 25 kg/m^2), occupational class (manual workers and non-manual employees), alcohol consumption (non-drinkers, low, moderate, high), cigarette smoking(<10 pack-years, 10-19 pack-years and ≥20 pack-years) (except for **study I**), silica (rock-drilling, stone crushing or stone dust, yes or no) (for **study II and IV**), and recruitment time period (1996-2006 and 2006-2014).

7.5 STATISTICAL ANALYSIS

The association(s) between exposure(s) and outcome(s) was/were assessed by calculating the odds ratios (OR) and 95% confidence interval(CI) using logistic regression. The exposures and outcomes for each study were shown in **table 6**. Both conditional and unconditional logistic regression were performed, but the results from both ways of analysis did not differ substantially. The final results shown in the results section were obtained using unconditional logistic regression with adjustment for the matching variables (age, sex and residential area), since this alternative generated results with greater precision. Adjustments for potential confounding factors were performed by adding the potential confounder as an additional covariate into the model.

Table 6. Outcome and Exposure for Study I to Study IV		
	Outcome	**Exposure**
Study I	ACPA-positive RA among male	silica (yes/no) smoking (current, past)
Study II	RA(overall) ; ACPA-positive RA; ACPA-negative RA	7 types of PW
Study III	anti-CII positive RA; anti-CII negative RA	6 types of PW
Study IV	RA(overall); ACPA-positive RA; ACPA-negative RA	cold cold outdoor cold indoor

The potential presence of interaction between two risk factors (A and B) was performed in **study I**, **study II** and **study IV**. Interaction on the additive scale was estimated by calculating the attributable proportion due to interaction (AP) as described by Rothman et al.[87] A brief description of AP calculation is shown below.

$AP=RERI/RR_{11}$, where $RERI = RR_{11}-RR_{10}-RR_{01}+1$

RERI corresponds to the relative excess risk due to interaction. RR_{11} refers to the relative risk among subjects exposed to both risk factors A and B. RR_{10} refers to the relative risk among subjects exposed to risk factor A but not B, and RR_{01} denotes the relative risk among subjects exposed to risk factor B but not A. Each relative risk is calculated using those who were unexposed to both risk factors as the reference group.

All analyses were performed using the SAS software package, V.9.4 (SAS Institute, Cary, North Carolina, USA).

7.5.1 Study I

Odds ratios (ORs) and 95% confidence intervals (95%CI) for the development of ACPA-positive RA associated with two exposures (silica and current smoking or silica and past smoking) were calculated using unconditional logistic regression, adjusting for the matching variables (age and residential area). Those who were both never smokers and unexposed to silica were used as the reference group. Additional adjustments including alcohol consumption (non-drinkers, low, moderate, high), education (university degree, yes/no) and body mass index (<25 kg/m^2 or $\geqslant$ 25 kg/m^2) did not substantially alter the ORs and were therefore not retained in the final analyses.

The interaction between silica exposure and pack-years of smoking (from 1 to 40 pack-years) and between silica exposure and years of smoking cessation (from 5 to 30 years), were evaluated based on the principle of departure from additivity of effects.[87]

7.5.2 Study II

For each type of physical workload, the odds of developing RA(overall), ACPA-positive RA and ACPA-negative RA among the exposed group was compared with the corresponding odds in the unexposed group. Separate analyses were performed for exposure status at baseline and exposure status at five years before baseline. Aside from the potential confounders described above, the 7 types of physical workload exposure were also considered as potential confounding factors for each other in this study.

We further examined whether exposure to more than one type of physical workload would increase the risk of developing RA. The subjects were categorized into 7 groups. Group 1(reference group) corresponds to those who reported they were not exposed to any of the physical workloads involved. Group 2, 3, 4, 5 and 6 corresponds to those who reported they were exposed to 1 type, 2 types, 3 types, 4 types, 5 types and 6 types of physical workload, respectively. Exposure to precision work was excluded from this analysis because the OR observed between exposure to precision work and the outcome was close to null. A p-value for trend was obtained by treating the categorical variable (group 1-6) as a continuous variable in the logistic regression model.

Interaction between HLA-DRB1 SE genes and different types of physical workload exposures at 5 years before baseline was performed, with regards to the risk of developing ACPA-positive RA.

7.5.3 Study III

For each type of physical workload, the odds of developing anti-CII positive RA or anti-CII negative RA was compared between the exposed group and the unexposed group (reference group). In this study, only cases recruited between 1996-2006 were included because the anti-CII level of cases recruited after 2006 are not yet measured. Controls included in this study were recruited from 1996 to 2014. A separate analysis in which both cases and controls were recruited from 1996-2006 was also done (data not shown). The results from this separate analysis had wider confidence intervals but relatively similar point estimates when compared to the final results shown in this study.

7.5.4 Study IV

The odds of developing RA (overall), ACPA-positive RA and ACPA-negative RA among those who reported they had worked in the cold environment (ever, current or past exposure) were compared with those who reported they had never worked in the cold environment (reference group).

The dose-response relationship between exposure to cold indoor work environment and risk of developing RA was investigated. The effect of duration, intensity, cumulative dose and cessation of working in cold indoor environment on the risk of developing RA were analyzed. Duration was quantified by the number of years working in cold indoor environment. Intensity was quantified by the number of hours per week. Cumulative dose was quantified by the number of work-years, which was calculated by multiplying duration with intensity. One work-year was equivalent to 2080 hours. Cessation was defined as the number of years the subjects had stopped working in the cold indoor environment.

Several cut-off methods were used for categorizing number of years, number of hours per week and number of work-years. Arbitrary cut-off values as well as cut-off values based on the median and tertile values of the exposed controls were used. The trends were relatively similar across these three ways of categorization, but only arbitrary cut-off values with four categories were presented in the final results. P- values for trend were obtained by treating the categorical variable as a continuous covariate in the logistic regression model.

Interaction between exposure to cold work environment and different types of physical workload was performed. We have collected information on physical workload exposure for two time points (i.e. baseline and five-years before baseline). In contrast, participants were asked to report two specific time periods in which they were exposed to cold work environment. Since there was difference in the temporal information on these two risk factors, two different methods of defining exposure to cold work environment were used. The first method was restricting both cold work environment exposure and physical workload exposure at 5 years before baseline. The second method was restricting only physical workload exposure at 5 years before baseline, while working in cold environment exposure was not restricted at any specific time point.

8 RESULTS

8.1 SMOKING-SILICA INTERACTION AND RISK OF RA

In addition to the previous finding on the interaction between cigarette smoking and silica, results from **study I** showed that this interaction depended on both the dose of smoking and the length of smoking cessation. The magnitude of the estimated silica-smoking interaction increase yhd as the pack-year of smoking increased, with the highest AP observed for smoking ⩾28 pack-years (AP=0.7 (95%CI: 0.4-0.9)) (**figure 2**). The interaction between silica and smoking among ex-smokers who ceased smoking ⩽10 years ago (AP=0.5 (95%CI: 0.1-0.9)) was relatively similar to the silica-smoking interaction observed among current smokers (AP=0.5 (95%CI: 0.2-0.8)). The interaction between silica and smoking cessation was no longer statistically significant after >10 years of smoking cessation (AP=0.1 (95%CI, -0.4-0.7)) (**figure 3**).

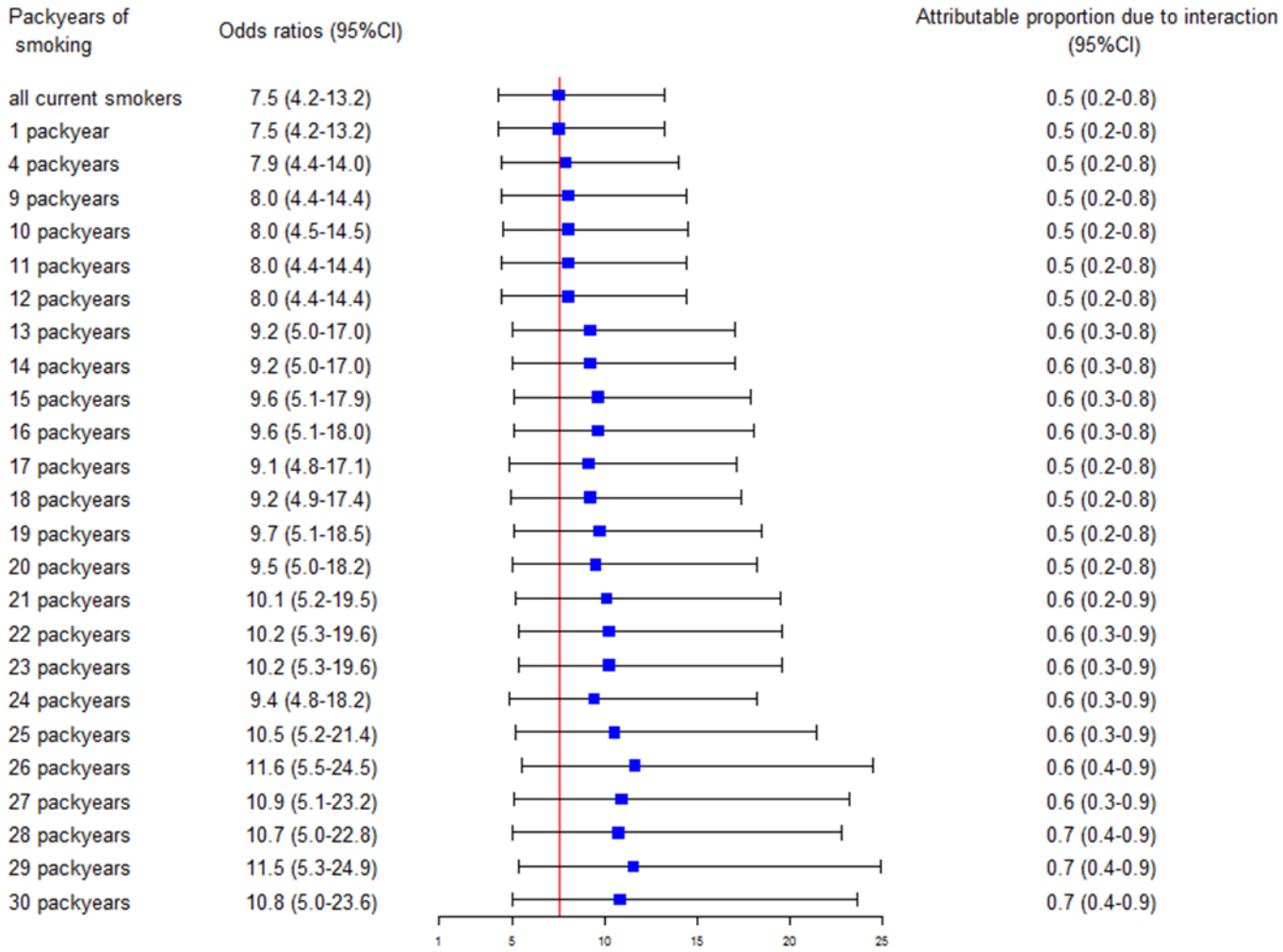

Figure 2. Interaction between smoking and silica exposure regarding risk of ACPA-positive RA among males, by pack-years of smoking

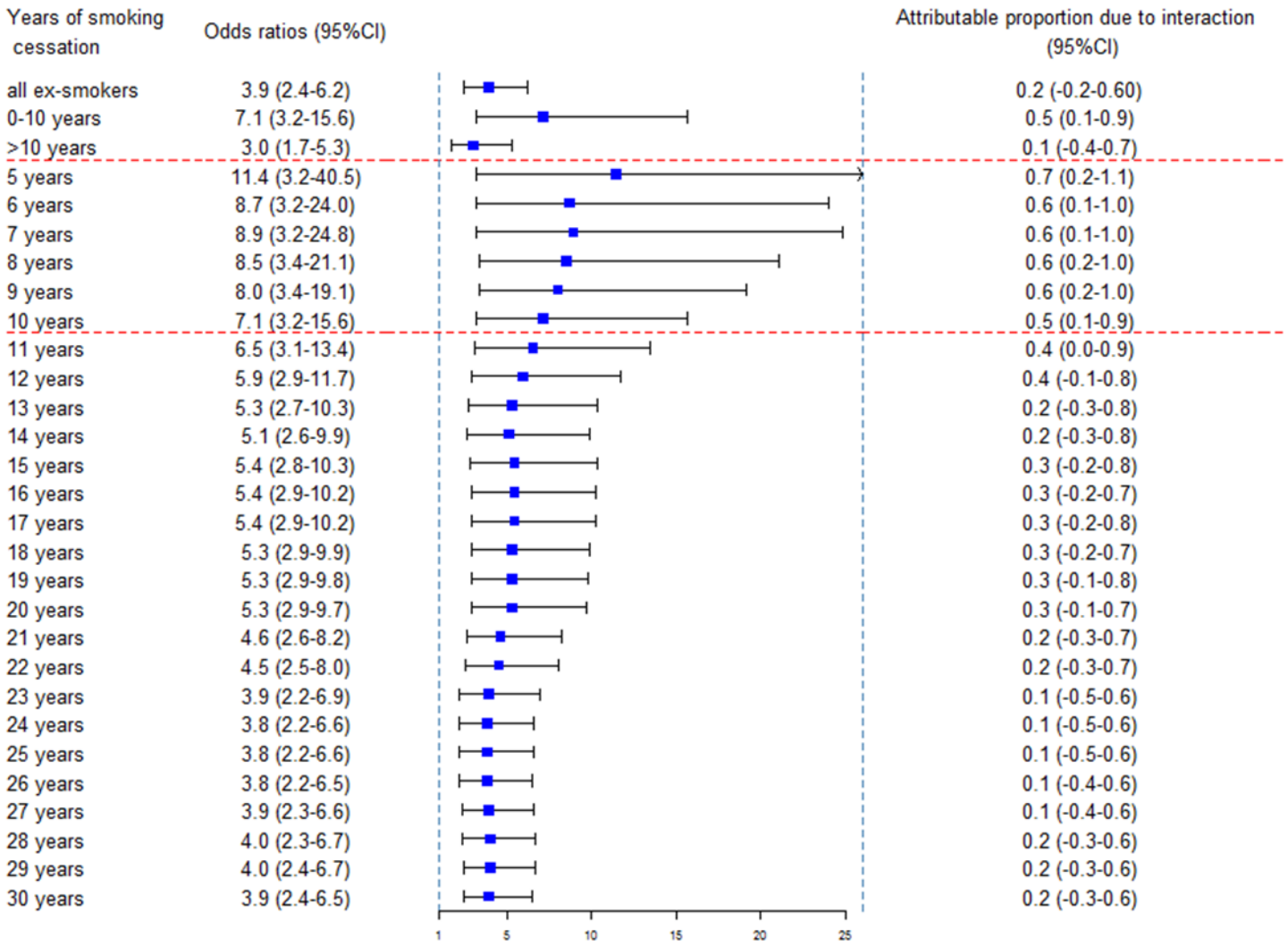

Figure 3. Interaction between smoking cessation and silica exposure regarding risk of ACPA-positive RA among males, by duration of smoking cessation

8.2 PHYSICAL WORKLOAD AND RISK OF RA

Out of seven different types of occupational physical workload, six were associated with an increased risk of developing RA. ORs observed for these 6 types of physical workload ranged from 1.3 (95%CI: 1.1-1.4) to 1.8(95%CI: 1.6-2.0) (**figure 4**). The highest OR was observed for exposure to hands above shoulder level. Exposure to precision work was the only type of physical workload observed to be not associated with the risk of developing RA (1.1 (95%CI: 1.0-1.3)). Exposure to more types of physical workload was observed to be associated with higher risk of developing RA (p-value for trend <0.0001) (**figure 5**).

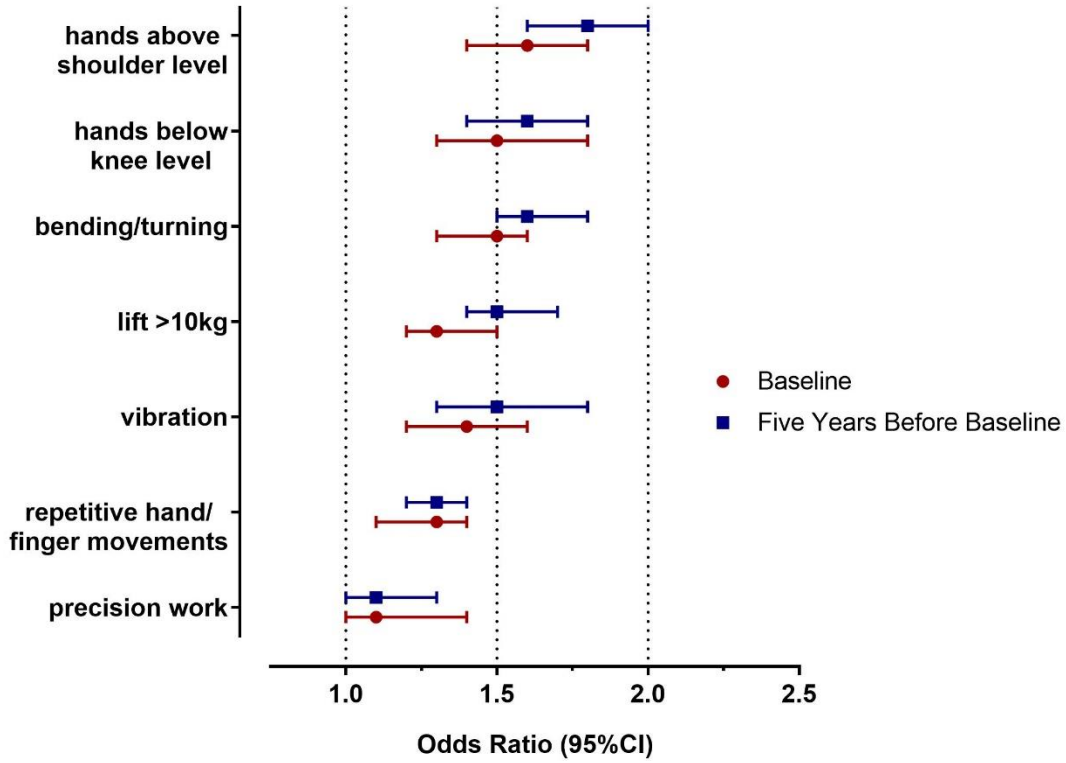

Figure 4. Odds ratio of developing rheumatoid arthritis among participants exposed to physical workload compared to unexposed.

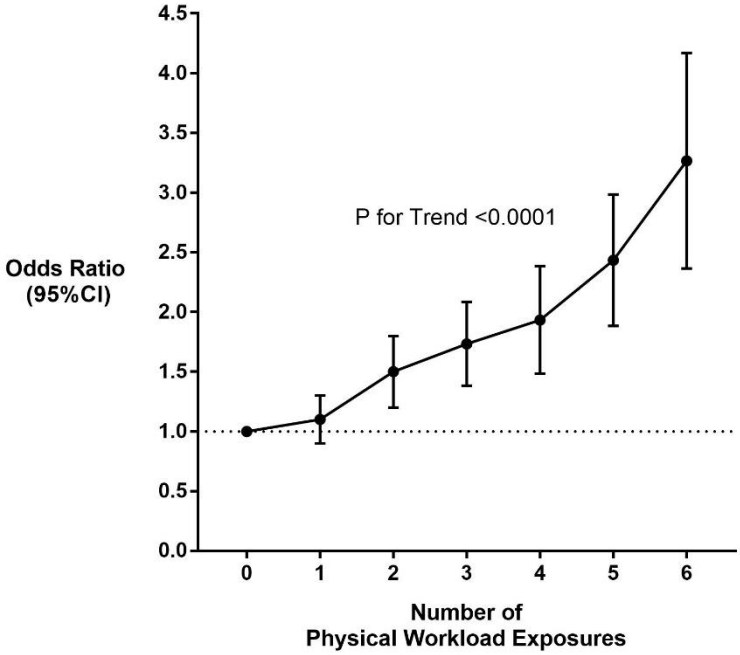

Figure 5. Odds ratio of developing RA across groups exposed to increasing number of types of physical workload compared to unexposed.

8.2.1 Physical Workload and Antibody Status

The six types of physical workload observed to be associated with the risk of RA (overall) were also observed to be associated with both ACPA-positive and ACPA-negative RA. The ORs observed for ACPA-positive RA and ACPA-negative RA were relatively similar, except for precision work. The ORs for ACPA-positive RA ranged from OR=1.2 (95%CI: 1.1-1.4) to OR=1.8 (95%CI: 1.6-2.0), while the ORs for ACPA-negative RA ranged from 1.4 (95%CI: 1.2-1.6) to 1.7(95%CI: 1.5-2.0).

These 6 types of physical workload were also found to be associated with an increased risk of both anti-CII positive and anti-CII negative RA, even though the ORs observed for anti-CII positive had wide confidence intervals **(figure 6)**.

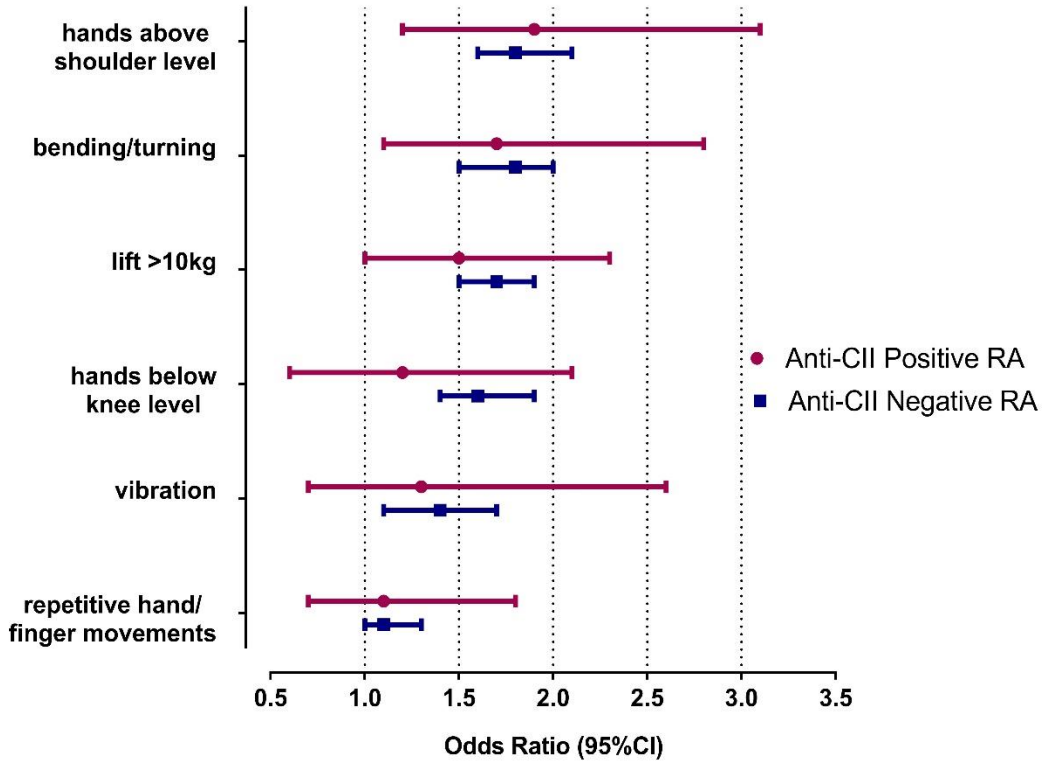

Figure 6. Odds ratio of developing anti-CII positive RA and anti-CII negative RA among participants exposed to physical workload compared to unexposed.

8.2.2 Physical Workload and HLA-DRB1

With regards to the risk of developing ACPA-positive RA, interaction between HLA-DRB1 shared epitope and physical workload (except precision work) was observed. The AP values ranged from 0.3(95%CI, 0.0-0.5) to 0.4 (95%CI, 0.2-0.5) (**figure 7**).

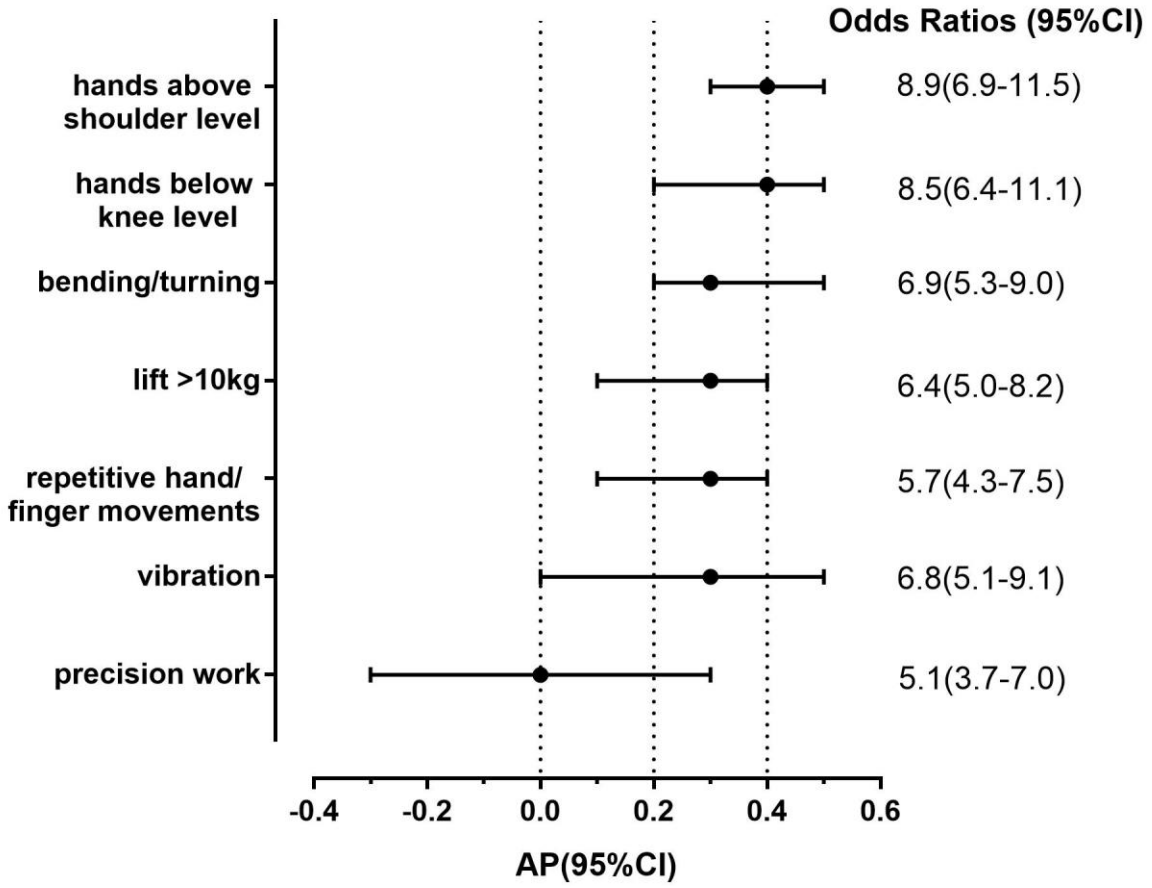

Figure 7. Attributable proportion due to interaction (AP) between physical workload and the HLA-DRB1 shared epitope. Odds ratios correspond to the odds ratios associated with the risk of developing ACPA-positive RA among subjects with HLA-DRB1 shared epitope and exposed to physical workload compared to those unexposed to both factors.

8.3 WORKING IN COLD ENVIRONMENT AND RISK OF RA

Working in cold environment was observed to be associated with an increased risk of developing RA (OR=1.5; 95%CI 1.4-1.7) (**figure 8**). Exposure to both indoor and outdoor cold work environment increased the risk of RA, but a dose-response relationship was observed only for exposure to cold indoor work. No major difference was found between the risk for two RA subtypes, namely ACPA-positive RA and ACPA-negative RA. Interaction (AP=0.3; 95%CI 0.1-0.5) was found between exposure to cold work environment and exposure to repetitive hand/finger movements at work.

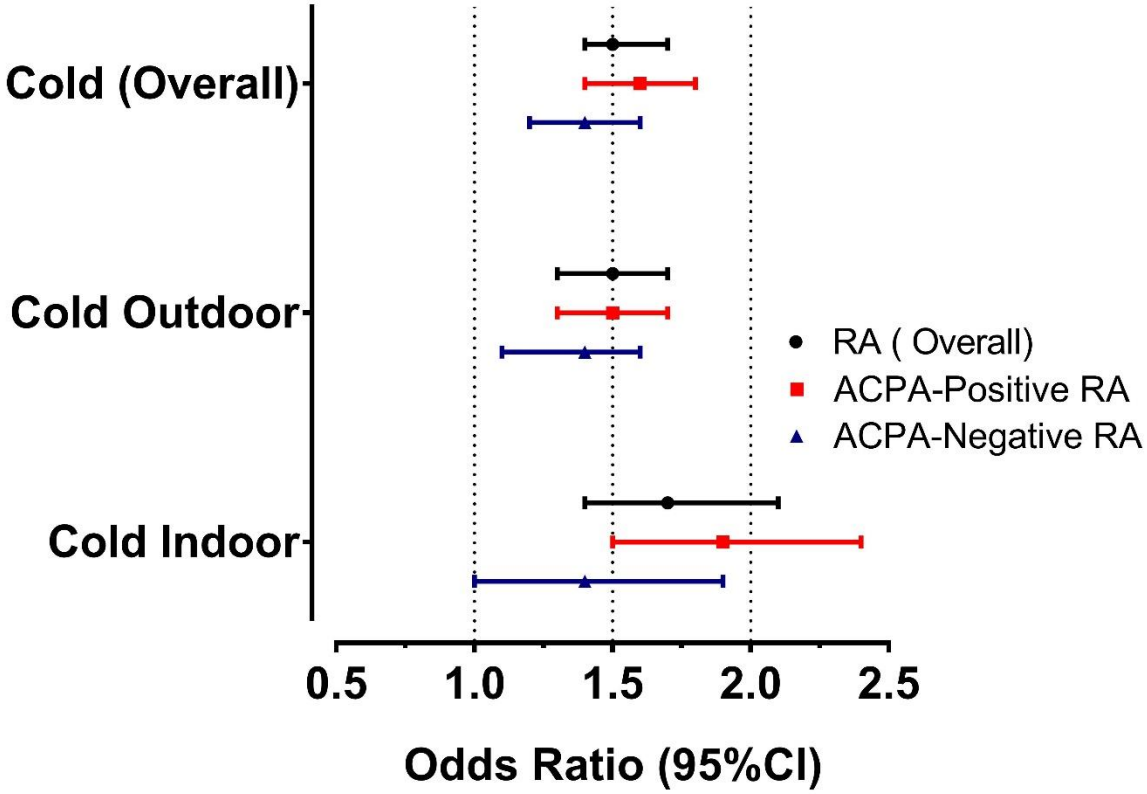

Figure 8. Odds ratios of developing RA among subjects worked in cold environment, cold indoor environment and cold outdoor environment.

9 DISCUSSION

9.1 MAIN FINDINGS AND IMPLICATIONS

In **study I** we observed that the effect of silica-smoking interaction with regards to the risk of ACPA-positive RA depended on the dose of smoking and it could take more than 10 years of smoking cessation for the silica-smoking interaction effect to disappear. The results strengthen the hypothesis that airborne exposures affecting the lungs play a role in the etiology of ACPA-positive RA. The findings also strengthen the rationale to advice both the public and those who are exposed to silica due to their occupation to avoid smoking.

In **study II** we found that physical workload is associated with the risk of developing both ACPA-positive and ACPA-negative RA. This study identified physical workload as a potential new environmental risk factor for RA. Prior to this study, physical workload has not been shown to be associated with RA, but has been known as a risk factor of osteoarthritis.[63,64] Osteoarthritis is a non-autoimmune joint disease. In contrast, RA is a more severe chronic disease in which excessive and uncontrolled inflammatory responses lead to self-destruction of human body tissues by the immune system. The involvement of the immune system in RA has resulted in immense number of researches focusing mainly on the immune aspects of RA etiology, while mechanical factors that directly affect the joints have generally been considered as unlikely contributing factors. The findings of **study II** highlight the importance of considering physical workload exposure as a risk factor not only for non-autoimmune joint disease such as osteoarthritis, but also for inflammatory diseases such as RA.

Study III was performed as an attempt to identify plausible biological mechanisms that could explain the association between physical workload and the risk of RA. We hypothesized that physical workload could injure the cartilage of the joints leading to immune reaction and production of antibodies against type-II collagen. However, no evidence was found in **study III** that suggests physical workload is associated with the development of anti-CII antibodies in RA. The findings may imply that the presence of anti-CII antibodies in RA is not due to lesions on the cartilage caused by mechanical forces on the joints around 5 years before disease diagnosis.

In **study IV**, we observed that working in cold environment is associated with the risk of developing RA. Whether cold environment has a role in causing RA disease is a commonly asked question and a concern from many RA patients. The findings from this study provide clues for this question, which has been lingered in human history for a long time. This study underscores the importance of taking the effect of cold environment on human physiological or immunity into considerations when studying the etiology of RA.

9.2 POTENTIAL BIOLOGICAL MECHANISMS

No studies in the current literature have investigated the potential biological mechanisms underlying the association between physical workload or exposure to cold work environment and risk of developing RA. Nevertheless, the following hypothesized biological mechanisms are probably worth investigating in the future.

Prolonged or repetitive postures or forces may exert excessive mechanical stress on the joints and the synovium. Mechanical stress could lead to protein denaturation. Under normal condition, the cells restore the denatured protein into their normal functional forms through upregulation of chaperones or heat shock proteins (HSP). Several heat shock proteins such as HSP60, HSP70 and HSP96 have been shown to be associated with RA.[88-90] Interestingly, HSP-70 have been shown to be overexpressed after shear stress stimulation of RA synovial tissue.[89] Based on the above-mentioned information, cellular dysregulations related to the disruption of the balance between protein denaturation rate and protein restoration rate by excessive mechanical stress could probably lead to chronic immune activation and diseases such as RA.

Citrullination of peptides is considered as one of the pathogenic event that leads to the establishment of RA.[91] Consequently, it is pivotal to understand what environmental factors trigger citrullination or other post-translational modifications leading to chronic inflammation. Since we observed that physical workload and cold work environment are associated with not only ACPA-positive RA but also ACPA-negative RA. This may imply that the exposures, physical workload and cold work environment, are probably not direct triggers of citrullination.

Some researchers postulate that chronic inflammation in the pathogenesis of RA, particularly ACPA-positive RA, is not initiated in the joints but in other organs such as the lungs.[91,92] It is hypothesized that some primary environmental exposures such as smoking could initiate citrullination in the lungs, which leads to the production of ACPAs and systemic immunity.[92] As the systemic immunity proceeds, some secondary environmental exposures reach/hit the joints, which leads to lesions and localized chronic inflammation in the joints.[93] Therefore, another potential biological mechanism that can explain the association between physical workload and risk of RA is: probably physical workload acts as the so-called “second-hit” in the course of RA disease pathogenesis. Although no evidence was found in **study III** to support the hypothesis that physical workload could induce lesions on the cartilage leading to anti-CII production, this hypothesis may still be worth investigating as there might be lesions on other components of the joints at another pre-RA time point.

In **study IV**, a positive interaction was observed between repetitive hand/finger movement and working in cold environment with regards to the risk of developing RA.

Working in cold environment demands higher workload from the muscles, tendons and joints than working in normal condition. Human physiological responses to cold environment include shivering and vasoconstriction. Shivering augments heat production while vasoconstriction maintains the core body temperature and decreases the temperature on the skin and the joint.[74] Lower temperature in the joint area may increase the viscosity of the synovial fluid,[94] which may require extra force and energy to move the joints and lead to a higher risk of injury or higher protein denaturation rate in the cellular level. Moreover, the functions of immune cells depend on the availability of oxygen and nutrients. Exposure to cold environment might decrease blood circulation and supply of oxygen and nutrients to the fingers and joints, which are relatively far from the core body and are in demand of oxygen and nutrients to perform physical workload. Under such situation, hypoxia might occur in the joint extremities leading to chronic inflammation and RA.

The causal relationship between RA and homeostasis of microbiome is still under investigation. A study reported that the microbiome in the gut and mouth of RA patients differs substantially from healthy controls.[95] Another study has observed that physical exercise increases the gut microbial diversity in elite athletes (i.e. 40 professional rugby union players versus 46 more sedentary controls).[96] It seems plausible that physical workload and cold work environment might also be associated with the homeostasis of gut microbiome, which may have a role in modulating immune response leading to RA.

These are only a few examples of possible hypotheses that can be generated to guide future researches on RA etiology. When conducting future researches on RA etiology, taking the perspectives and experiences of RA patients as well as modifiable environmental risk factors into consideration would facilitate the translation of scientific findings into applicable preventive strategies.

9.3 STUDY DESIGN

9.3.1 Strengths

All four studies in this thesis are based on the EIRA study, a population based case-control study, in which incident cases in a defined geographical area are identified and controls are drawn randomly from the population at risk. This enhances the generalizability of the findings. All cases are incident and the time duration from the appearance of first disease symptom to diagnosis is relatively short (mean=10 months). In addition, only exposures prior to the appearance of the first disease symptom were considered and analyzed. These increase our confidence that the exposure we studied happened before disease onset, which complies with our main research aim of identifying risk factors for developing RA. Many potential confounding factors could be taken into considerations in all the analyses, owing to the possibility of retrieving information on many environmental/lifestyle factors

through questionnaires. The response rate for cases (94%) and controls (77%) are relatively high when compared with the general response rate of case-control studies in the literature. Nevertheless, as the participation proportion is higher among the cases than controls, potential selection bias cannot be ignored.

9.3.2 Limitations

9.3.2.1 Selection Bias

The main concern of selection bias is connected to the 23% non-participation proportion among the controls. If these non-participating controls were likely to be those who were either exposed to physical workload or exposed to cold work environment(i.e. if the probability to be included in the study is associated with exposure), then the observed ORs in our studies would be overestimations.

A previous study had acquired information on EIRA non-participants from registry data and observed that non-participation is associated with socioeconomic class.[97] This observation offers one possible strategy to investigate the degree of such selection bias by performing stratification analysis based on educational level or occupational class and examine whether the risk estimates differ across strata. By employing this strategy, we observed that the ORs are robust across strata and we concluded that this selection bias is unlikely to have substantial impact on the observed associations.

Another possible strategy to investigate the potential influence of such selection bias on our results is by conducting an "educated guess analysis". We could calculate the limits of selection bias (1) by presuming that the 23% non-participating controls have similar exposure frequency as compared with the exposure frequency of the participating cases; (2) by finding the threshold exposure frequency of the non-participating control in order for the OR to be equal to null effect or 1.0. For example, the observed OR for hands above shoulder level is 1.8 (95%CI, 1.6-2.0) and the observed exposure frequency of the participating cases is 30%. If we assume that the exposure frequency of the non-participating controls is also 30%, the estimated OR would be 1.5 (95% CI, 1.4-1.7). Based on the given example, we observed that even when the non-participating controls have similar exposure frequency as the participating cases, the positive association between physical workload and risk of RA would still remain statistically significant. Secondly, we also observed that the exposure frequency of the non-participating controls should be unreasonably higher than the exposure frequency of the cases in order for the OR to reach 1.0 (**table 7**). This further strengthen our deduction that the observed positive association between physical workload or cold work environment and risk of RA is unlikely due to such selection bias.

Table 7. Exposure frequency of participating cases and controls and theoretical threshold frequency of non-participating controls			
Exposures	**Observed Exposure Frequency**		**Threshold Frequency**
	participating cases	*participating controls*	*non-participating controls*
Repetitive Bending/turning	67%	55%	>90%
repetitive hand/finger movements	71%	66%	>80%
lift more than 10kg	56%	45%	>80%
hands below knee level	23%	16%	>40%
hands above shoulder level	30%	20%	>60%
vibration	19%	14%	>30%
working in the cold	23%	18%	>40%

9.3.2.2 Misclassification of Exposures

The exposure information is self-reported and collected through questionnaire. Physical workload and exposure to cold environment are two exposures that may be suspected by RA patients as possible factors causing their disease. Consequently, the cases may recall these exposures differently than the non-cases. This recall bias could lead to differential exposure misclassification and overestimated odds ratios.

In the constituent papers of this thesis, we had investigated the likelihood of this potential recall bias. With regards to the physical workload exposure, we argued that the exposure we analyzed was categorized into a binary variable (i.e. exposed versus unexposed). This limits the influence of recall bias, because it is less likely for cases to over-report their exposure status (yes/no) than to over-report their exposure frequency and duration. As for the cold work environment exposure, we compared the occupations between the exposed cases and exposed controls. Since the occupations are comparable between these two groups, we concluded that the observed positive associations are unlikely due to such potential recall bias.

An alternative method to investigate the strength of this potential recall bias is by conducting a multidimensional bias analysis.[98] In this bias analysis, we would like to examine how the estimated odd ratio would change across different sensitivity values assigned for the exposure classification method used in the study. We assumed that the specificity for both the cases and controls is relatively high (approximately 90%), because if a subject who is truly unexposed to physical workload, it is unlikely for that subject to report it as exposed. In contrast, the sensitivity among the cases and controls is likely to be different, and likely to be higher for the cases than for the controls.

Given the presumptions above, a multidimensional bias analysis was performed for the exposure working with hands above shoulder level and the results are shown in **figure 9**. Based on the observed bias adjusted ORs, our observed positive association could be an overestimation if the sensitivity among the controls is lower than 40% and the sensitivity among the cases is above 70%, conditioning with a specificity of 90% for both cases and controls. Few studies have reported the sensitivity and specificity of physical workload questionnaire; nevertheless, the reported sensitivity and specificity of dichotomous questionnaire information on physical workload are relatively high, with sensitivity of around 70%-90% and specificity of around 70%-100%.[85,99] Notably these sensitivity and specificity were measured based on a population without a specific disease. Given this information, we could deduce that the sensitivity for the controls in our study is likely to be greater than 50%, and the adjusted ORs with a sensitivity of higher than 50% in the controls suggest a positive association (**figure 9**). Multidimensional bias analysis was also performed for other physical workload exposures (data not shown). In brief, the adjusted ORs observed for hands below knee level and vibration demonstrate a similar trend as the OR observed for hands above shoulder level. The adjusted ORs observed for other types of physical workload reveal a higher probability of either no association or negative association.

			Cases								
Controls		Se	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
Se	Sp	Sp	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
0.9	0.9		2.41	2.89	3.61	4.80	7.16	14.12	493.45	-14.98	-7.38
0.8	0.9		2.07	2.48	3.10	4.12	6.15	12.12	423.40	-12.85	-6.33
0.7	0.9		1.73	2.07	2.58	3.44	5.13	10.11	353.36	-10.73	-5.28
0.6	0.9		1.38	1.66	2.07	2.76	4.11	8.11	283.32	-8.60	-4.24
0.5	0.9		1.04	1.25	1.56	2.07	3.10	6.10	213.28	-6.47	-3.19
0.4	0.9		0.70	0.84	1.05	1.39	2.08	4.10	143.24	-4.35	-2.14
0.3	0.9		0.36	0.43	0.54	0.71	1.06	2.09	73.20	-2.22	-1.09
0.2	0.9		0.02	0.02	0.02	0.03	0.05	0.09	3.16	-0.10	-0.05
0.1	0.9		-0.33	-0.39	-0.49	-0.65	-0.97	-1.91	-66.88	2.03	1.00

Figure 9. The adjusted odds ratios across different sensitivities of exposure (i.e. hands above shoulder level) classification among cases and controls. Adjusted odds ratios highlighted in red are impossible values given the data and the combination of values assigned to the sensitivity and specificity. Se denotes sensitivity; Sp denotes specificity.

Although the multidimensional bias analysis provides more information on the potential impact of recall bias, it has some limitations. Firstly, the assigned values for sensitivity and specificity are based on educated guessing and there is no information available in the literature that gives a reliable sensitivity and specificity values for our data. Secondly, it does not provide information regarding which of the bias adjusted ORs is most likely to occur. The second limitation can be overcome through the use of probabilistic bias analysis; however, the accuracy of probabilistic bias analysis depends substantially on the chosen probabilistic distributions used to calculate the adjusted OR and these calculations are beyond the scope of this thesis.[98]

9.3.2.3 Misclassification of Disease

RA is considered as a clinical syndrome that encompasses several disease subsets, which may have distinct inflammatory mechanistic pathways that result into a common clinical feature of persistent synovial inflammation and destruction of bone and cartilage.[100] Despite the complexity of RA, classification criteria for RA are developed, which facilitates research on RA. The RA cases in **study I-IV** were classified based on the ACR 1987 or ACR 2010 criteria and were diagnosed by rheumatologists. Although ACR 2010 criteria has greater sensitivity in identifying early RA patients as compared with ACR 1987 criteria, only a very small proportion of cases (0.1%) fulfilled only the ACR 2010 but not ACR 1987. Consequently, the emergence of the ACR 2010 criteria may have an influence on identifying RA patients at an earlier stage, but would not result in differential misclassification of disease in this thesis.

There is a possibility that some cases are misdiagnosed, but this likelihood is considered small since all cases were diagnosed by a rheumatologist. Even though accessibility to health care is free of charge in Sweden, some cases in the study area may not have been included. Based on our experience, this is mainly due to administrative reasons such as lack of time or change of personnel and among others. It seems unlikely that subjects who were exposed to silica, cigarette smoke, physical workload or cold work environment would have a different tendency in seeking medical care than those who were not exposed to these exposures. The proportion of invited cases that answered the questionnaire was high (94%). Overall, the likelihood that the exposures investigated in this thesis would influence the probability for a case to be included in the study is considered to be relatively small.

9.3.2.4 Confounding

A confounder is a factor that is associated with both the exposure and the outcome, but should not be an effect of the exposure nor the outcome. All studies in this thesis have taken many potential confounding factors (as listed in section 7.4) into consideration. However, similar to many other observational studies, residual confounding due to unmeasured confounding factors or due to imperfect information on the measured confounding factors cannot be ruled out. Presence of a bias due to confounding could, in principle, reverse or obscure the observed associations in this thesis.

In this thesis, we found a positive association between physical workload and risk of RA. Other occupational factors such as occupational noise, air pollution and shift work are possible potential confounding factors that were not adjusted. A Finnish study reported that physical workload, occupational noise and shift work have a tendency to co-occur in the same occupational groups.[101] Recently, a study conducted by Hedström et al. using EIRA study material,[102] has found that permanent night shift work has a protective effect on RA

development, whereas rotating and day-oriented shift work increased the risk of ACPA-positive RA but not ACPA-negative RA. Likewise, this study also did not adjust for physical workload. Given this information, adjusting for permanent night shift work might increase the OR observed for physical workload and risk of RA. Adjusting for rotating and day-oriented shift work might change the OR observed for physical workload and ACPA-positive RA towards null but not for physical workload and ACPA-negative RA.

In **study IV**, we found an association between working in cold environment and risk of RA. The exposure in this study is self-reported and rather subjective. Many potential confounding factors that are closely related to the subjective feeling of cold have not been taken into consideration. A few example of these unmeasured potential confounding factors are temperature, barometric pressure, humidity, unknown dietary habits and unknown physiological characteristics. Although these unmeasured factors are known to be related to the exposure (i.e. working in cold environment), their associations with the outcome (i.e. risk of developing RA) have not been studied, and are thus unknown. Nonetheless, as these unmeasured potential confounding factors were not taken into account in the analysis, we could not distinguish whether subjective feeling of cold or other relevant elements such as temperature or humidity is more relevant to the development of RA.

9.4 STATISTICAL ESTIMATES

The odds ratios derived from case-control study can sometimes mirror the incidence rate ratios derived from a cohort study performed in the same study base. This depends on how the controls are sampled from the study base.[103] When controls are drawn from the source population in the beginning of the follow-up time, then the odds ratio approximates risk ratio, since the controls provide an estimate of the exposure prevalence among persons at risk. When controls are drawn from the source population at the end of the observation period, then the odds ratio is approximately equal to rate ratio if the outcome is rare. When controls are drawn from the source population at the same time as a case arise (i.e. density sampling), and a control may at later time become a case, then the odds ratio will provide an approximate estimate of the incidence rate ratio, which is similar to what would have been observed in a cohort study conducted on the same study base. This is because the controls selected using such incidence density sampling technique provides an estimate of the ratio of exposed to unexposed person-time. In the EIRA study, at each time a case is identified, controls are randomly selected from the Swedish population register. Accordingly, the odds ratios reported in our studies, in principle, estimate the incidence rate ratio of developing RA, conditioning on absence of substantial selection bias among controls.

Attributable proportion due to interaction (AP) was used to evaluate the potential presence of biological interaction between two risk factors. AP is calculated based on the principle of departure from additivity of effect. According to this principle, if the risk among those who are exposed to both two risk factors is greater than the sum of the individual risks

conferred by each of the two factors in the absence of the other, then this provides evidence of sufficient cause interaction.[87,104] In **study IV**, an AP value of 0.3 was observed for the interaction between exposure to cold work environment and repetitive hand/finger movements. Presuming that these factors are causal, this AP value would suggest that around 30% of those who were exposed to both cold work environment and repetitive hand/finger movements developed RA, because both these two risk factors co-occur in mechanisms leading to the disease. This not only gives insight on the etiology of RA, but also has important public-health implications. In **study II**, the AP value between exposure to hands above shoulder level and having the shared epitope gene was observed to be 0.4 regarding the risk of developing ACPA-positive RA. This implies that 40% of the ACPA-positive cases that emerged among those who have the shared epitope gene and were exposed to hands above shoulder level could have been prevented, if these subjects had avoided the exposure hands above shoulder level.

9.5 SIGNIFICANCE

For experimental researches, the strength of evidence partly depends on whether the hypothesis is verified through in vitro or in vivo experiments. The experiments performed on living organisms such as mice are usually perceived as more valid than experiments performed using cells. However, scientific knowledge acquired through mice experiments are not always applicable to humans. To ensure that scientific research results from the lab can be applied to humans, clinical or epidemiological research are needed. On the other hand, epidemiological research results would be more convincing if there are available experimental evidence that support the causal relationship it investigates.

In this thesis, **study I** strengthened the research hypothesis that airway exposure such as cigarette smoke and silica are critical for the etiology of ACPA-positive RA. The causal relationship between airway exposure and RA has been investigated in many studies.[92,105-107] In contrast, in **study II** and **study IV**, we have identified two new environmental risk factors for RA, namely physical workload and working in cold environment. Since these findings are relatively new, supporting experimental data are in demand to support the underlying causal relationship. As an attempt to provide a mechanistic explanation to these findings from epidemiology, we investigated the relationship between physical workload and presence of anti-CII in RA, but we did not find evidence suggesting an association between anti-CII antibodies and RA (**study III**). In short, the significance of this thesis is not to confirm whether physical workload or cold work environment is a cause of RA, but rather it provides clues on two new risk factors for RA and forms the foundation for future interventional or experimental studies for the analysis of causation.

Future research can focus on validating the findings of this thesis using other study populations, or using other methods to assess the exposure such as using a job exposure matrix instead of information from self-reported questionnaire. Future research may also

focus on drawing plausible cellular etiologic pathways and test them using laboratory experiments.

9.6 CONCLUSION

The findings of this thesis not only further confirm that lung exposures(e.g. cigarette smoke and silica) play an important role in the etiology of ACPA-positive RA, but also identified two new environmental risk factors for RA, namely physical workload and cold work environment. Whether physical workload and cold work environment are contributing factors to RA development has previously not been investigated in systematically designed studies with enough statistical power and represents a frequently asked question from RA patients. This thesis has provided an unprecedented piece of scientific evidence to support this unaddressed concern. In addition, the results are also valuable for creating relevant etiologic hypotheses concerning the disease etiology of RA which can be tested using interventional or experimental studies.

9.7 REFLECTION ON RESEARCH ETHICS

The benefits of conducting the epidemiologic studies in my research project outweigh the risks. My research projects provide new knowledge on modifiable risk factors for RA. The gained knowledge may contribute to better understanding of the natural history of RA, to RA prevention and to improved health care. Unlike intervention studies, the studies of my research project does not involve invasive procedures done on patients. However, my studies carry a potential risk of breaching personal data which may harm participants' integrity. This would happen (1) when personal identity data are accessed by unauthorized persons; (2)when data are presented in a very detailed manner in which the identity of certain study subjects become traceable; and (3) when the data are misused politically. If breach of confidentiality happened, it would damage not only the integrity of the participants but also the trust the participants have on scientific research, which would impair healthcare research and development.

To safeguard the integrity of participants, proper storage and handling of data is required. The data in EIRA study are stored and analyzed in a data-security computer system, in which unauthorized persons are unable to access. This prevents possible leakage of data in case the computers in which the analyses were performed are lost. The researchers performing the analyses (e.g. myself) are blinded from the Swedish personal identity number of all participants; thus, preventing any data misuse by any researcher analysing the data. Lastly, the result of the data were presented in a comprehensive and summarized manner in which no specific participants are potentially identifiable based on the scientific manuscripts or other relevant publications.

Ethical permits were received from the Karolinska Institutet Ethics Committee and the Regional Stockholm Ethics Committee (Dnr 96-174, Dnr 2006/476-31/4) for conducting the EIRA study. Written informed consent was received from all participants of the EIRA study. The EIRA study adheres to the Swedish Privacy Protection Law for protecting confidential information of the participants.

10 ACKNOWLEDGEMENT

Supervisors and Colleagues

I would like to express my gratitude to all my supervisors for giving me the opportunity (or perhaps more precisely "opportunities") to accomplish my PhD study at Karolinska Institutet. I appreciate the freedom, trust and quality-oriented scientific supervision they gave throughout this PhD. I am grateful for their advice, kindness and understanding especially during those tough times when I stumbled. I have learned some basic scientific knowledge and acquired some survival skills in this field. Thank you.

I feel lucky to have the chance to work with Anna Ilar, my close colleague and friend, her positive attitude had certainly increased the odds of finding happiness in this PhD journey. I would also like to thank German Dario Carrasquilla Lopez for sharing laughters in the office; Boel Brynedal and Helga Westerlind for their generousity in sharing knowledge and initiatives in creating research meeting opportunities; Max Vikström for all statistical lessons and discussions; Rebecka Hjort for all the educational epidemiological discussions; Anna Peterson for all the sophisticated administrative help; and many more colleagues at IMM who have contributed to a friendly and comfortable working environment.

EIRA Study Group

Big thanks to the EIRA administrative group (Marie-Louise Serra, Lena Nise, Edit Ekström, Caroline Colliander, Ida Palmqvist and many more) and all EIRA participants. Without their work, the projects in this thesis would be impossible.

Thanks to Lena Nise for introducing me to the EIRA research group on my first day at IMM and for providing all kinds of help from sending mails to many complicated questions about EIRA dataset and for being my first SAS teacher.

Family and Mentor

I feel blessed to have all the supports from my family. I am and will always be grateful to my kind and inspiring mentor Alasdair Gibb, who has given me invaluable fatherly guidance and unconditional support.

Karolinska Institutet and European Union

I am privileged to receive PhD education at Karolinska Institutet. I am immensely thankful for the free and unlimited access to all sources of knowledge such as libraries,

scientific publications and courses. If there were no free courses in statistics and epidemiology, it would be very difficult for me to do a PhD in epidemiology. Thank you to all the taxpayers in Sweden who made all these amazing things possible. I am also thankful to the European FP7 Marie Curie Initial Training Network Osteoimmune which provided funding for this PhD education training.

11 REFERENCES

1 McInnes, I. B. & Schett, G. The pathogenesis of rheumatoid arthritis. *The New England journal of medicine* **365**, 2205-2219, doi:10.1056/NEJMra1004965 (2011).

2 Young, A. & Koduri, G. Extra-articular manifestations and complications of rheumatoid arthritis. *Best Practice & Research Clinical Rheumatology* **21**, 907-927, doi:http://dx.doi.org/10.1016/j.berh.2007.05.007 (2007).

3 Listing, J. *et al.* Mortality in rheumatoid arthritis: the impact of disease activity, treatment with glucocorticoids, TNFalpha inhibitors and rituximab. *Annals of the rheumatic diseases* **74**, 415-421, doi:10.1136/annrheumdis-2013-204021 (2015).

4 Avina-Zubieta, J. A., Thomas, J., Sadatsafavi, M., Lehman, A. J. & Lacaille, D. Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies. *Annals of the rheumatic diseases* **71**, 1524-1529, doi:10.1136/annrheumdis-2011-200726 (2012).

5 Smolen, J. S., Aletaha, D. & McInnes, I. B. Rheumatoid arthritis. *Lancet (London, England)* **388**, 2023-2038, doi:10.1016/s0140-6736(16)30173-8 (2016).

6 Arnett, F. C. *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis and rheumatism* **31**, 315-324 (1988).

7 Banal, F., Dougados, M., Combescure, C. & Gossec, L. Sensitivity and specificity of the American College of Rheumatology 1987 criteria for the diagnosis of rheumatoid arthritis according to disease duration: a systematic literature review and meta-analysis. *Annals of the rheumatic diseases* **68**, 1184-1191, doi:10.1136/ard.2008.093187 (2009).

8 Combe, B. Early rheumatoid arthritis: strategies for prevention and management. *Best practice & research. Clinical rheumatology* **21**, 27-42, doi:10.1016/j.berh.2006.08.011 (2007).

9 van Dongen, H. *et al.* Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis: a double-blind, randomized, placebo-controlled trial. *Arthritis and rheumatism* **56**, 1424-1432, doi:10.1002/art.22525 (2007).

10 Aletaha, D. *et al.* 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis and rheumatism* **62**, 2569-2581, doi:10.1002/art.27584 (2010).

11 Smolen, J. S. *et al.* EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. *Annals of the rheumatic diseases* **73**, 492-509, doi:10.1136/annrheumdis-2013-204573 (2014).

12 Smolen, J. S. & Aletaha, D. Rheumatoid arthritis therapy reappraisal: strategies, opportunities and challenges. *Nature reviews. Rheumatology* **11**, 276-289, doi:10.1038/nrrheum.2015.8 (2015).

13 Felson, D. T. & Klareskog, L. The genetics of rheumatoid arthritis: new insights and implications. *Jama* **313**, 1623-1624, doi:10.1001/jama.2015.1710 (2015).

14 van Boekel, M. A., Vossenaar, E. R., van den Hoogen, F. H. & van Venrooij, W. J. Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. *Arthritis research* **4**, 87-93, doi:10.1186/ar395 (2002).

15 De Rycke, L. *et al.* Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. *Annals of the rheumatic diseases* **63**, 1587-1593, doi:10.1136/ard.2003.017574 (2004).

16 Girbal-Neuhauser, E. *et al.* The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various

sites of (pro)filaggrin by deimination of arginine residues. *Journal of immunology (Baltimore, Md. : 1950)* **162**, 585-594 (1999).
17 Schellekens, G. A., de Jong, B. A., van den Hoogen, F. H., van de Putte, L. B. & van Venrooij, W. J. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *The Journal of clinical investigation* **101**, 273-281, doi:10.1172/jci1316 (1998).
18 Klareskog, L. *et al.* A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. *Arthritis and rheumatism* **54**, 38-46, doi:10.1002/art.21575 (2006).
19 Nishimura, K. *et al.* Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. *Annals of internal medicine* **146**, 797-808 (2007).
20 Rantapaa-Dahlqvist, S. *et al.* Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis and rheumatism* **48**, 2741-2749, doi:10.1002/art.11223 (2003).
21 Nielen, M. M. *et al.* Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis and rheumatism* **50**, 380-386, doi:10.1002/art.20018 (2004).
22 van der Helm-van Mil, A. H., Verpoort, K. N., Breedveld, F. C., Toes, R. E. & Huizinga, T. W. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis research & therapy* **7**, R949-958, doi:10.1186/ar1767 (2005).
23 Klareskog, L., Ronnelid, J., Lundberg, K., Padyukov, L. & Alfredsson, L. Immunity to citrullinated proteins in rheumatoid arthritis. *Annual review of immunology* **26**, 651-675, doi:10.1146/annurev.immunol.26.021607.090244 (2008).
24 Sophia Fox, A. J., Bedi, A. & Rodeo, S. A. The basic science of articular cartilage: structure, composition, and function. *Sports health* **1**, 461-468, doi:10.1177/1941738109350438 (2009).
25 Holmdahl, R. *et al.* Type II collagen autoimmunity in animals and provocations leading to arthritis. *Immunological reviews* **118**, 193-232 (1990).
26 Manivel, V. A. *et al.* Anticollagen type II antibodies are associated with an acute onset rheumatoid arthritis phenotype and prognosticate lower degree of inflammation during 5 years follow-up. *Annals of the rheumatic diseases*, doi:10.1136/annrheumdis-2016-210873 (2017).
27 Frisell, T., Saevarsdottir, S. & Askling, J. Family history of rheumatoid arthritis: an old concept with new developments. *Nature reviews. Rheumatology* **12**, 335-343, doi:10.1038/nrrheum.2016.52 (2016).
28 Frisell, T. *et al.* Familial risks and heritability of rheumatoid arthritis: role of rheumatoid factor/anti-citrullinated protein antibody status, number and type of affected relatives, sex, and age. *Arthritis and rheumatism* **65**, 2773-2782, doi:10.1002/art.38097 (2013).
29 Okada, Y. *et al.* Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* **506**, 376-381, doi:10.1038/nature12873 (2014).
30 Padyukov, L., Silva, C., Stolt, P., Alfredsson, L. & Klareskog, L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. *Arthritis and rheumatism* **50**, 3085-3092, doi:10.1002/art.20553 (2004).
31 Raza, K. & Gerlag, D. M. Preclinical Inflammatory Rheumatic Diseases: An Overview and Relevant Nomenclature. *Rheumatic Disease Clinics of North America* **40**, 569-580, doi:https://doi.org/10.1016/j.rdc.2014.07.001 (2014).

32 Jick, S. S., Choi, H., Li, L., McInnes, I. B. & Sattar, N. Hyperlipidaemia, statin use and the risk of developing rheumatoid arthritis. *Annals of the rheumatic diseases* **68**, 546-551, doi:10.1136/ard.2008.091967 (2009).
33 Shadick, N. A. *et al.* Low-dose aspirin in the primary prevention of rheumatoid arthritis: the Women's Health Study. *Arthritis care & research* **62**, 545-550, doi:10.1002/acr.20042 (2010).
34 Walitt, B. *et al.* Effects of postmenopausal hormone therapy on rheumatoid arthritis: the women's health initiative randomized controlled trials. *Arthritis and rheumatism* **59**, 302-310, doi:10.1002/art.23325 (2008).
35 Machold, K. P. *et al.* The Stop Arthritis Very Early (SAVE) trial, an international multicentre, randomised, double-blind, placebo-controlled trial on glucocorticoids in very early arthritis. *Annals of the rheumatic diseases* **69**, 495-502, doi:10.1136/ard.2009.122473 (2010).
36 Karlson, E. W. *et al.* Association of environmental and genetic factors and gene-environment interactions with risk of developing rheumatoid arthritis. *Arthritis care & research* **65**, 1147-1156, doi:10.1002/acr.22005 (2013).
37 Gabriel, S. E. & Michaud, K. Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. *Arthritis research & therapy* **11**, 229, doi:10.1186/ar2669 (2009).
38 van Vollenhoven, R. F. Sex differences in rheumatoid arthritis: more than meets the eye. *BMC medicine* **7**, 12, doi:10.1186/1741-7015-7-12 (2009).
39 Tobon, G. J., Youinou, P. & Saraux, A. The environment, geo-epidemiology, and autoimmune disease: Rheumatoid arthritis. *Autoimmunity reviews* **9**, A288-292, doi:10.1016/j.autrev.2009.11.019 (2010).
40 McDougall, C., Hurd, K. & Barnabe, C. Systematic review of rheumatic disease epidemiology in the indigenous populations of Canada, the United States, Australia, and New Zealand. *Semin Arthritis Rheum* **46**, 675-686, doi:10.1016/j.semarthrit.2016.10.010 (2017).
41 Eriksson, J. K. *et al.* Incidence of rheumatoid arthritis in Sweden: a nationwide population-based assessment of incidence, its determinants, and treatment penetration. *Arthritis care & research* **65**, 870-878, doi:10.1002/acr.21900 (2013).
42 Eriksson, J. K., Johansson, K., Askling, J. & Neovius, M. Costs for hospital care, drugs and lost work days in incident and prevalent rheumatoid arthritis: how large, and how are they distributed? *Annals of the rheumatic diseases* **74**, 648-654, doi:10.1136/annrheumdis-2013-204080 (2015).
43 Costenbader, K. H., Feskanich, D., Mandl, L. A. & Karlson, E. W. Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. *The American journal of medicine* **119**, 503.e501-509, doi:10.1016/j.amjmed.2005.09.053 (2006).
44 Kallberg, H. *et al.* Smoking is a major preventable risk factor for rheumatoid arthritis: estimations of risks after various exposures to cigarette smoke. *Annals of the rheumatic diseases* **70**, 508-511, doi:10.1136/ard.2009.120899 (2011).
45 Karlson, E. W. *et al.* A retrospective cohort study of cigarette smoking and risk of rheumatoid arthritis in female health professionals. *Arthritis and rheumatism* **42**, 910-917, doi:10.1002/1529-0131(199905)42:5<910::aid-anr9>3.0.co;2-d (1999).
46 Stolt, P. *et al.* Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Annals of the rheumatic diseases* **62**, 835-841 (2003).
47 Jiang, X., Alfredsson, L., Klareskog, L. & Bengtsson, C. Smokeless tobacco (moist snuff) use and the risk of developing rheumatoid arthritis: Results from the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) case-control study. *Arthritis Care Res (Hoboken)*, doi:10.1002/acr.22325 (2014).

48 Di Giuseppe, D., Orsini, N., Alfredsson, L., Askling, J. & Wolk, A. Cigarette smoking and smoking cessation in relation to risk of rheumatoid arthritis in women. *Arthritis research & therapy* **15**, R56, doi:10.1186/ar4218 (2013).
49 Criswell, L. A. *et al.* Cigarette smoking and the risk of rheumatoid arthritis among postmenopausal women: results from the Iowa Women's Health Study. *The American journal of medicine* **112**, 465-471 (2002).
50 Olsson, A. R., Skogh, T., Axelson, O. & Wingren, G. Occupations and exposures in the work environment as determinants for rheumatoid arthritis. *Occupational and environmental medicine* **61**, 233-238 (2004).
51 Blanc, P. D., Jarvholm, B. & Toren, K. Prospective risk of rheumatologic disease associated with occupational exposure in a cohort of male construction workers. *The American journal of medicine* **128**, 1094-1101, doi:10.1016/j.amjmed.2015.05.001 (2015).
52 Parks, C. G., Conrad, K. & Cooper, G. S. Occupational exposure to crystalline silica and autoimmune disease. *Environmental health perspectives* **107 Suppl 5**, 793-802 (1999).
53 Stolt, P. *et al.* Silica exposure is associated with increased risk of developing rheumatoid arthritis: results from the Swedish EIRA study. *Annals of the rheumatic diseases* **64**, 582-586, doi:10.1136/ard.2004.022053 (2005).
54 Stolt, P. *et al.* Silica exposure among male current smokers is associated with a high risk of developing ACPA-positive rheumatoid arthritis. *Annals of the rheumatic diseases* **69**, 1072-1076, doi:10.1136/ard.2009.114694 (2010).
55 Arkema, E. V., Karlson, E. W. & Costenbader, K. H. A prospective study of periodontal disease and risk of rheumatoid arthritis. *The Journal of rheumatology* **37**, 1800-1804, doi:10.3899/jrheum.091398 (2010).
56 Eriksson, K. *et al.* Prevalence of Periodontitis in Patients with Established Rheumatoid Arthritis: A Swedish Population Based Case-Control Study. *PLoS One* **11**, e0155956, doi:10.1371/journal.pone.0155956 (2016).
57 Rosell, M., Wesley, A. M., Rydin, K., Klareskog, L. & Alfredsson, L. Dietary fish and fish oil and the risk of rheumatoid arthritis. *Epidemiology (Cambridge, Mass.)* **20**, 896-901, doi:10.1097/EDE.0b013e3181b5f0ce (2009).
58 Jin, Z., Xiang, C., Cai, Q., Wei, X. & He, J. Alcohol consumption as a preventive factor for developing rheumatoid arthritis: a dose-response meta-analysis of prospective studies. *Annals of the rheumatic diseases* **73**, 1962-1967, doi:10.1136/annrheumdis-2013-203323 (2014).
59 Orellana, C. *et al.* Oral contraceptives, breastfeeding and the risk of developing rheumatoid arthritis: results from the Swedish EIRA study. *Annals of the rheumatic diseases* **76**, 1845-1852, doi:10.1136/annrheumdis-2017-211620 (2017).
60 Salliot, C., Bombardier, C., Saraux, A., Combe, B. & Dougados, M. Hormonal replacement therapy may reduce the risk for RA in women with early arthritis who carry HLA-DRB1 *01 and/or *04 alleles by protecting against the production of anti-CCP: results from the ESPOIR cohort. *Annals of the rheumatic diseases* **69**, 1683-1686, doi:10.1136/ard.2009.111179 (2010).
61 Doran, M. F., Crowson, C. S., O'Fallon, W. M. & Gabriel, S. E. The effect of oral contraceptives and estrogen replacement therapy on the risk of rheumatoid arthritis: a population based study. *The Journal of rheumatology* **31**, 207-213 (2004).
62 Orellana, C. *et al.* Postmenopausal hormone therapy and the risk of rheumatoid arthritis: results from the Swedish EIRA population-based case-control study. *European journal of epidemiology* **30**, 449-457, doi:10.1007/s10654-015-0004-y (2015).

63 Vingard, E., Alfredsson, L. & Malchau, H. Osteoarthrosis of the hip in women and its relation to physical load at work and in the home. *Annals of the rheumatic diseases* **56**, 293-298 (1997).
64 Coggon, D. *et al.* Osteoarthritis of the hip and occupational lifting. *American journal of epidemiology* **147**, 523-528 (1998).
65 Mikkonen, P. *et al.* Physical workload and risk of low back pain in adolescence. *Occupational and environmental medicine* **69**, 284-290, doi:10.1136/oemed-2011-100200 (2012).
66 Tseng, C. M. *et al.* The effect of cold temperature on increased exacerbation of chronic obstructive pulmonary disease: a nationwide study. *PLoS One* **8**, e57066, doi:10.1371/journal.pone.0057066 (2013).
67 Hyrkas, H., Jaakkola, M. S., Ikaheimo, T. M., Hugg, T. T. & Jaakkola, J. J. K. Asthma and allergic rhinitis increase respiratory symptoms in cold weather among young adults. *Respir. Med.* **108**, 63-70, doi:10.1016/j.rmed.2013.10.019 (2014).
68 Skandfer, M., Talykova, L., Brenn, T., Nilsson, T. & Vaktskjold, A. Low back pain among mineworkers in relation to driving, cold environment and ergonomics. *Ergonomics* **57**, 1541-1548, doi:10.1080/00140139.2014.904005 (2014).
69 Burstrom, L., Jarvholm, B., Nilsson, T. & Wahlstrom, J. Back and neck pain due to working in a cold environment: a cross-sectional study of male construction workers. *Int. Arch. Occup. Environ. Health* **86**, 809-813, doi:10.1007/s00420-012-0818-9 (2013).
70 Bhaskaran, K. *et al.* Effects of ambient temperature on the incidence of myocardial infarction. *Heart* **95**, 1760-1769, doi:10.1136/hrt.2009.175000 (2009).
71 Manou-Stathopoulou, V. *et al.* The effects of cold and exercise on the cardiovascular system. *Heart* **101**, 808-820, doi:10.1136/heartjnl-2014-306276 (2015).
72 Hochstadter, E. F. & Ben-Shoshan, M. Cold-induced urticaria: challenges in diagnosis and management. *BMJ case reports* **2013**, doi:10.1136/bcr-2013-010441 (2013).
73 Simon, T. D., Soep, J. B. & Hollister, J. R. Pernio in pediatrics. *Pediatrics* **116**, E472-E475, doi:10.1542/peds.2004-2681 (2005).
74 Makinen, T. M. & Hassi, J. Health problems in cold work. *Industrial health* **47**, 207-220 (2009).
75 Kurppa, K., Viikari-Juntura, E., Kuosma, E., Huuskonen, M. & Kivi, P. Incidence of tenosynovitis or peritendinitis and epicondylitis in a meat-processing factory. *Scandinavian journal of work, environment & health* **17**, 32-37 (1991).
76 Sormunen, E., Remes, J., Hassi, J., Pienimaki, T. & Rintamaki, H. Factors associated with self-estimated work ability and musculoskeletal symptoms among male and female workers in cooled food-processing facilities. *Industrial health* **47**, 271-282 (2009).
77 Aasmoe, L., Bang, B., Egeness, C. & Lochen, M. L. Musculoskeletal symptoms among seafood production workers in North Norway. *Occupational medicine (Oxford, England)* **58**, 64-70, doi:10.1093/occmed/kqm136 (2008).
78 Savage, E. M. *et al.* Does rheumatoid arthritis disease activity correlate with weather conditions? *Rheumatology international* **35**, 887-890, doi:10.1007/s00296-014-3161-5 (2015).
79 Iikuni, N. *et al.* What's in season for rheumatoid arthritis patients? Seasonal fluctuations in disease activity. *Rheumatology (Oxford, England)* **46**, 846-848, doi:10.1093/rheumatology/kel414 (2007).
80 Smedslund, G., Mowinckel, P., Heiberg, T., Kvien, T. K. & Hagen, K. B. Does the weather really matter? A cohort study of influences of weather and solar conditions on daily variations of joint pain in patients with rheumatoid arthritis. *Arthritis and rheumatism* **61**, 1243-1247, doi:10.1002/art.24729 (2009).

81 Smedslund, G. & Hagen, K. B. Does rain really cause pain? A systematic review of the associations between weather factors and severity of pain in people with rheumatoid arthritis. *European journal of pain (London, England)* **15**, 5-10, doi:10.1016/j.ejpain.2010.05.003 (2011).
82 Guenel, P., Breum, N. O. & Lynge, E. Exposure to silica dust in the Danish stone industry. *Scandinavian journal of work, environment & health* **15**, 147-153 (1989).
83 Healy, C. B., Coggins, M. A., Van Tongeren, M., MacCalman, L. & McGowan, P. Determinants of respirable crystalline silica exposure among stoneworkers involved in stone restoration work. *The Annals of occupational hygiene* **58**, 6-18, doi:10.1093/annhyg/met045 (2014).
84 Hildebrandt, V. H., Bongers, P. M., van Dijk, F. J., Kemper, H. C. & Dul, J. Dutch Musculoskeletal Questionnaire: description and basic qualities. *Ergonomics* **44**, 1038-1055, doi:10.1080/00140130110087437 (2001).
85 Stock, S. R., Fernandes, R., Delisle, A. & Vezina, N. Reproducibility and validity of workers' self-reports of physical work demands. *Scandinavian journal of work, environment & health* **31**, 409-437 (2005).
86 Bot, S. D. *et al.* Internal consistency and validity of a new physical workload questionnaire. *Occupational and environmental medicine* **61**, 980-986, doi:10.1136/oem.2003.011213 (2004).
87 Rothman, K. J., Greenland, S. & Walker, A. M. Concepts of interaction. *American journal of epidemiology* **112**, 467-470 (1980).
88 Huang, Q. Q. *et al.* Heat shock protein 96 is elevated in rheumatoid arthritis and activates macrophages primarily via TLR2 signaling. *Journal of immunology (Baltimore, Md. : 1950)* **182**, 4965-4973, doi:10.4049/jimmunol.0801563 (2009).
89 Schett, G. *et al.* Enhanced expression of heat shock protein 70 (hsp70) and heat shock factor 1 (HSF1) activation in rheumatoid arthritis synovial tissue. Differential regulation of hsp70 expression and hsf1 activation in synovial fibroblasts by proinflammatory cytokines, shear stress, and antiinflammatory drugs. *The Journal of clinical investigation* **102**, 302-311, doi:10.1172/jci2465 (1998).
90 MacHt, L. M. *et al.* Relationship between disease severity and responses by blood mononuclear cells from patients with rheumatoid arthritis to human heat-shock protein 60. *Immunology* **99**, 208-214 (2000).
91 Malmstrom, V., Catrina, A. I. & Klareskog, L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. *Nat Rev Immunol* **17**, 60-75, doi:10.1038/nri.2016.124 (2017).
92 Catrina, A. I., Ytterberg, A. J., Reynisdottir, G., Malmstrom, V. & Klareskog, L. Lungs, joints and immunity against citrullinated proteins in rheumatoid arthritis. *Nature reviews. Rheumatology* **10**, 645-653, doi:10.1038/nrrheum.2014.115 (2014).
93 Perry, E., Kelly, C., Eggleton, P., De Soyza, A. & Hutchinson, D. The lung in ACPA-positive rheumatoid arthritis: an initiating site of injury? *Rheumatology* **53**, 1940-1950, doi:10.1093/rheumatology/keu195 (2014).
94 Hunter, J. Effects of cold on hand activities with special reference to joints and fluid viscosities. Protection and functioning of hands in cold climates. *National Academy of Sciences, NRC* (1957).
95 Zhang, X. *et al.* The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nature medicine* **21**, 895-905, doi:10.1038/nm.3914 (2015).
96 Barton, W. *et al.* The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level. *Gut*, doi:10.1136/gutjnl-2016-313627 (2017).

97 Bengtsson, C. *et al.* Non-participation in EIRA: a population-based case-control study of rheumatoid arthritis. *Scandinavian journal of rheumatology* **39**, 344-346, doi:10.3109/03009740903501634 (2010).
98 Lash, T. L., Fox, M. P. & Fink, A. K. *Applying Quantitative Bias Analysis to Epidemiologic Data*. 109-116 (Springer-Verlag New York, 2009).
99 Pope, D. P., Silman, A. J., Cherry, N. M., Pritchard, C. & Macfarlane, G. J. Validity of a self-completed questionnaire measuring the physical demands of work. *Scandinavian journal of work, environment & health* **24**, 376-385 (1998).
100 Scott, D. L., Wolfe, F. & Huizinga, T. W. Rheumatoid arthritis. *Lancet (London, England)* **376**, 1094-1108, doi:10.1016/s0140-6736(10)60826-4 (2010).
101 Virkkunen, H., Harma, M., Kauppinen, T. & Tenkanen, L. The triad of shift work, occupational noise, and physical workload and risk of coronary heart disease. *Occupational and environmental medicine* **63**, 378-386, doi:10.1136/oem.2005.022558 (2006).
102 Hedström, A. K., Åkerstedt, T., Klareskog, L. & Alfredsson, L. Relationship between shift work and the onset of rheumatoid arthritis. *RMD open* **3**, doi:10.1136/rmdopen-2017-000475 (2017).
103 Pearce, N. What does the odds ratio estimate in a case-control study? *International journal of epidemiology* **22**, 1189-1192 (1993).
104 VanderWeele, T. J. Sufficient cause interactions and statistical interactions. *Epidemiology (Cambridge, Mass.)* **20**, 6-13, doi:10.1097/EDE.0b013e31818f69e7 (2009).
105 Ytterberg, A. J. *et al.* Shared immunological targets in the lungs and joints of patients with rheumatoid arthritis: identification and validation. *Annals of the rheumatic diseases* **74**, 1772-1777, doi:10.1136/annrheumdis-2013-204912 (2015).
106 Demoruelle, M. K. *et al.* Anti-Citrullinated Protein Antibodies Are Associated With Neutrophil Extracellular Traps in the Sputum in Relatives of Rheumatoid Arthritis Patients. *Arthritis & rheumatology (Hoboken, N.J.)* **69**, 1165-1175, doi:10.1002/art.40066 (2017).
107 Demoruelle, M. K., Deane, K. D. & Holers, V. M. When and where does inflammation begin in rheumatoid arthritis? *Current opinion in rheumatology* **26**, 64-71, doi:10.1097/bor.0000000000000017 (2014).