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EPIDEMIOLOGICAL STUDIES IN MALAYSIA AND SWEDEN ON ASSOCIATIONS BETWEEN SMOKING, SILICA EXPOSURE, AND THE RISK OF DEVELOPING RHEUMATOID ARTHRITIS

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Stockholm 2012

Published by Karolinska Institutet. Printed by Larserics Digital Print AB

ISBN 978-91-7457-986-4

ABSTRACT

Rheumatoid arthritis (RA) is a chronic, inflammatory disease, frequently associated with joint destruction. Knowledge regarding the aetiology of RA is mainly generated on Caucasian populations. Less is known about RA in other populations with different genetic backgrounds and lifestyles. The aim of this thesis was to contribute to a better knowledge regarding the aetiology of RA in other populations, particularly Asian populations, by studying the association between airborne exposures and RA risk, here smoking and occupational exposure to silica. We studied the risk of developing RA by different subtypes of the disease, defined by the presence or absence of antibodies to citrullinated protein antigens (ACPA+ RA and ACPA- RA).

This thesis is mainly based on data from the Malaysian Epidemiological Investigation of Rheumatoid Arthritis (MyEIRA), Paper I, II and IV. MyEIRA is a population-based case-control study where cases and controls provided extensive information on lifestyle as well as occupational exposures. Cases and controls also provided blood samples for serological and genetic analysis. In Paper III, data from the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) population-based case control study was used. Information regarding the environmental exposures was gathered by means of a questionnaire. Cases and controls provided blood samples for genetic and serological analysis.

Our results in the MyEIRA study indicate that smokers had an increased risk of developing ACPA+ RA, but not ACPA- RA, compared with never-smokers. A significant dose-response relationship between cumulative dose of smoking (expressed by pack-years) and risk of ACPA+ RA was observed. A significant interaction was noted between smoking and the HLA-DRB1 shared epitope (SE) alleles in the risk of developing ACPA+ RA. We also found that the most common SE allele in the Asian population, HLA-DRB1*0405, also showed signs of interaction with smoking with regard to risk of ACPA+ RA.

We further studied the relationship between occupational exposure to silica, i.e. another airborne exposure, and the risk of developing RA in the Swedish EIRA study. Men that had been exposed to silica in their work were observed to have a moderately increased risk of ACPA+ RA but not ACPA- RA compared to men without such exposure. A significant interaction between silica exposure and current smoking was observed with regard to the risk of developing ACPA+ RA. The findings from MyEIRA were similar to those from EIRA; thus occupational exposure to silica was associated with an increased risk of developing ACPA+ RA, but not ACPA- RA. Furthermore, there were signs of interaction between silica and smoking with regard to risk of ACPA+ RA, even though small numbers hampered a firm conclusion.

In conclusion, this study shows that airborne environmental exposures are strongly associated with risk for RA in Malaysia. The results should have impact on efforts to prevent RA in this large part of the world, as well as for further comparative studies aimed at understanding the aetiology of RA in different populations.

LIST OF PUBLICATIONS

I. **YAHYA A**, Bengtsson C, Lai TC, Larsson PT, Mustafa AN, Abdullah NA, Muhamad N, Hussein H, Klareskog L, Alfredsson L, Shahnaz Murad: Smoking is associated with an increased risk of developing ACPA-positive but not ACPA-negative rheumatoid arthritis in Asian populations: evidence from the Malaysian MyEIRA case-control study. Mod Rheumatol (2012) 22:524–531.

II. Too CL*, **YAHYA A***, Murad S, Dhaliwal JS, Larsson PT, Muhamad NA, Abdullah NA, Mustafa AN, Klareskog L, Alfredsson L, Padyukov L, Bengtsson C. Smoking interacts with HLA-DRB1 shared epitope in the development of anti-citrullinated protein antibody-positive rheumatoid arthritis: results from the Malaysian Epidemiological Investigation of Rheumatoid Arthritis (MyEIRA). Arthritis Res Ther 2012, 14(2):R89. (*Contributed equally)

III. Stolt P, **YAHYA A**, Bengtsson C, Kallberg H, Ronnelid J, Lundberg I, Klareskog L, Alfredsson L. Silica exposure among male current smokers is associated with a high risk of developing ACPA-positive rheumatoid arthritis. Ann Rheum Dis. 2010 Jun; 69(6):1072-1076.

IV. **YAHYA A**, Bengtsson C, Larsson PT, Too CL, Mustafa AN, Abdullah NA, Muhamad NA, Klareskog L, Murad S, Alfredsson L, for MyEIRA study group. Silica is associated with an increased risk of developing ACPA-positive rheumatoid arthritis in an Asian population: Evidence from the Malaysian MyEIRA case-control study. Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

ACPA	Antibodies to citrullinated protein antigens
ACPA+	Antibodies to citrullinated protein antigens positive
ACPA-	Antibodies to citrullinated protein antigens negative
ACR	American College of Rheumatology
AP	Attributable proportion due to interaction
CI	Confidence interval
EIRA	Epidemiological Investigation of Rheumatoid Arthritis
HLA	Human Leukocyte antigen
MyEIRA	Malaysian Epidemiological Investigation of Rheumatoid Arthritis
OR	Odds ratio
RA	Rheumatoid arthritis
RF	Rheumatoid factor
SAS	Statistical Analysis System
SE	Shared epitope

1 INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory disease, which primarily affects synovial joints. The extended inflamed synovial erodes the articular cartilage and bone thus causing joint deformity, leading to progressive physical disability [1].

The exact aetiology of RA is unknown. Numerous studies have suggested that RA has a complex pathogenesis in which genes, environment and immunity act together in the development of the disease. The disease is more frequent among women than men and the prevalence as well as incidence increases with age [1-4].

It is uncertain how long RA has existed in Europe, Asia or Africa, and it has been suggested that RA became common only after the 17th century in these parts of the world. However, paleopathology of skeletons from North American Indians aged several thousand years showed evidence of RA.

The nomenclature of rheumatoid arthritis began in 1611 when Guilaume de Baillou recognized rheumatism as arthritis. Two centuries after that, a clear description of RA was derived by Augustin-Jacob Landré Beauvais in 1800. Six decades later, a British physician, Sir Alfred Bring Garrod (1817-1907), coined the term "rheumatoid arthritis" in 1859. Since then, there have been numerous attempts to rename the disease based on various 'observations and manifestation'. But not until 1922, did the British Ministry of Health formally accept "rheumatoid arthritis" as the official term. Nearly twenty years later, the term was adopted by the American Rheumatism Association [5].

1.1 THE PREVALENCE AND INCIDENCE OF RA

In industrialised countries, the prevalence of RA has been estimated to be between 0.5% and 1%. The occurrence of RA is believed to vary among different populations, and the majority of studies have been carried out in Northern Europe and North America. The variation in prevalence could be explained by environmental and genetic factors, but also by diverse disease classifications, the denominator used for prevalence, variability in the age of onset, study design, sample size and sampling method [6, 7].

In Europe, the prevalence of RA was found to vary between 0.2% and 1.1%, where southern Europe has the lowest prevalence between 0.2% and 0.7% [8-16]. In Sweden, the overall prevalence of RA in 2008 was estimated as 0.8%, 1.1% in women and 0.4% in men [4]. In the United States, the overall prevalence was estimated at about 0.6% [17] in which the North American Indians were observed to have the highest prevalence ranging between 1.4 and 7.1%, [18].

In contrast, information on the prevalence or incidence of RA in other parts of the world is scanty and limited. The estimated prevalence of RA for several regions relies mostly on cross sectional studies of WHO-ILAR COPCORD [19]. These studies reported that the prevalence of RA ranges between 0.1% in Thailand and about 0.5% in India [20]. An urban-rural difference was also observed in some studies indicating higher prevalence in urban than rural areas [21].

1.2 MAIN AUTOANTIBODIES IN RHEUMATOID ARTHRITIS

Rheumatoid factor (RF) was first described as an autoantibody about 70 years ago (1940) by Emil Waaler. RF was the first auto-reactive element discovered in the sera of RA patients and was found in about 60-80% of adult RA patients [22, 23]. RF could also be found in other autoimmune diseases such as Sjögren's syndrome, infectious diseases (e.g. hepatitis, tuberculosis) and also to some extent among healthy people which makes its specificity limited [23]. Despite its moderate specificity, a high level of RF, especially at the onset, is associated with aggressive and destructive types of disease [24]. Thus, RF is used as one of the diagnostic and prediction markers for RA [25].

More recently, another set of autoantibodies called anti-citrullinated protein antibodies (ACPA), usually measured by anti-cyclic citrullinated protein antibodies (anti-CCP), has become a centre of attention in RA. The reactivity was first described in the 1960s by Nienhuis et al, where the reactivity was discovered to be against the citrullinated proteins in the late 1990s, and measurements of these antibodies has become essential in recent years [26] [27]. ACPA is a group of autoantibodies that recognise citrullinated epitopes [28]. Citrulline is a non-standard amino acid that is formed from posttranslational modification of arginine residue in the proteins mediated by peptidylarginine deiminase (PAD) enzymes [29]. ACPA have a very high specificity for RA (95%) and sensitivity similar to that of RF (68%) [30]. Higher titre level of ACPA was found to be associated with disease activity and joint erosion [31, 32]. That development of ACPA preceded the onset of RA was discovered by a group of researchers from northern Sweden. They found that ACPA could be detected several years before the onset of RA [33]. Its presence together with HLA-DRB1 locus antigens was found to be highly associated with the future onset of RA [34].

1.3 DIAGNOSIS AND TREATMENT OF RHEUMATOID ARTHRITIS

Lack of exclusive symptoms for RA is a concern with regard to diagnosis. RA normally shares similar symptoms with other forms of polyarthritis such as psoriatic arthritis. Several established classification criteria for RA have been developed, i.e. the 1958 American Rheumatism Association (ARA) criteria, the Rome criteria in 1961, the New York criteria in 1966, the 1987 American College of Rheumatology (ACR) criteria for RA and recently the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010 Classification Criteria to facilitate the classification of the disease [25, 35-37]. Normally, patients present with symptoms such as fatigue, pain and stiffness, tenderness and swollenness in multiple joints, particularly the hand joints. Morning stiffness is also a common feature [38, 39]. There is so far no single diagnostic test to confirm RA. The major basis for the diagnosis is the clinical examination, but also several laboratory tests are of value for the diagnosis, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), complete blood cell count and ACPA [40].

The main aim of the treatment for RA is to prevent irreversible joint deformity and to eliminate inflammation and other disease-related signs that reduce quality of life for the patients. The common strategy nowadays is to treat RA aggressively as early as possible after diagnosis. Evidence has shown that aggressive treatment in the early stage can improve quality of life in a long time perspective, and more specifically prevent damage to the bone [41]. The treatment strategies can be tailored to reducing pain, reducing bone erosion and improving joint functionality [42]. Nonsteroidal anti-inflammatory drug (NSAID), disease modifying antirheumatic drug (DMARD), biologics and steroids are the most common pharmacological therapies recommended to RA patients. Appropriate combinations of different drugs and early treatments have shown to be the most efficient way to achieving a good response to therapy and remission of disease activity [43].

1.4 RISK FACTORS

A risk factor is any factor that increases a risk of developing a disease. Risk factors could be environmental and/or genetic. Decades of studies have led to a consensus that RA is a multifactorial disease, where genetic and environmental risk factors acting in concert lead to its occurrence and expression [44]. Environmental and life style factors such as smoking, diet, obesity, socioeconomic factors, infectious agents, hormonal factors, blood transfusion, traffic pollution and certain occupational exposures, for example silica and mineral oil have been associated with the development of RA [6, 7, 45-65]. Yet, several environmental factors such as moderate alcohol intake and oral contraceptive (OC) use were found to have an inverse association with the risk of RA [59-61, 66]. In this thesis, we will focus on environmental exposures related to the lungs, namely smoking and occupational exposure to silica together with a specific genetic factor, the HLA-DRB1 shared epitope (SE) alleles and their influence on the risk of developing RA.

1.5 ENVIRONMENTAL EXPOSURES

1.5.1 Smoking

Smoking is the most studied environmental risk factor for RA so far. The association between smoking and RA was first observed unintentionally during the study of oral contraceptive use and rheumatoid arthritis by Vessey et al in 1987 [48]. Since then, smoking has gradually become accepted as an environmental risk factor for the development of RA, predominantly among RF-positive RA and those with ACPA [47, 49, 52, 53, 67-71]. According to a finding from the Swedish EIRA study, smoking increases about twofold the risk for ACPA+ RA and leads to a more pronounced risk (up to 21 fold) among those with a specific genetic profile, called human leukocyte antigen (HLA)-DRB1 shared epitope (SE) [72]. The association between smoking and risk of ACPA+ RA is further supported by the finding of a higher presence of citrullinated proteins in bronchoalveolar lavage cells among smokers than in non-smokers [72]. According to the same study, there seems to be no association between smoking and risk of ACPA negative RA [72]. These findings have subsequently been replicated in US and Dutch populations [73, 74].

The knowledge generated so far regarding the combined influence of genetic variants, smoking, other environmental factors and the risk for RA has so far been derived mainly from studies among Caucasian populations. Much less is known in other populations with different lifestyles and genetic backgrounds, for example in Asian populations. Thus, it is as yet uncertain whether, and if so, to what extent smoking is a risk factor for RA in Asian populations, and in particular to which extent similar mechanisms are involved as in Caucasian populations and in Western societies. For example, a study from Korea from 2010 has indicated that smoking in combination with HLA-DRB1 SE increases the risk for both ACPA+ RA and ACPA- RA [71].

1.5.2 Silica

Another potential modifiable risk factor for RA is occupational exposure to silica dust. Silica or crystalline silica dust is abundant in sand, rock and soil. Exposure to a high level of respirable silica dust can cause chronic inflammation in lungs and could lead to fibrosis in the lungs and other organs [75]. Previous studies found that exposure to silica increased the risk for autoimmune diseases such as rheumatoid arthritis, systemic sclerosis, systemic lupus, Sjögren's syndrome and chronic renal disease [75-78].

A meta-analysis in 2002 by Khuder et al found that exposure to silica significantly increased the risk for RA among exposed men compared to men unexposed to silica [64]. However, it was impossible to evaluate the dose-response relationship due to lack of reliable information on cumulative silica exposure. Further, an analysis using death certificates from 27 states of the United States observed a significantly increased risk for silicosis, chronic obstructive pulmonary disease (COPD), pulmonary tuberculosis and rheumatoid arthritis. In this study, a significant non-linear trend in risk with increasing silica exposure was observed only for RA [79]. A study conducted in southern Sweden also observed evidence of a dose-response relation for exposure to stone or silica among men [51]. In 2005, the Swedish EIRA study found that occupational exposure to silica among men increased the risk for developing RA, even when smoking habits are taken into account [80]. The results were compatible with a synergistic effect of smoking and silica, but did not allow a firm conclusion on this because of the small numbers of silica-exposed never smokers. Although there are many epidemiological studies that show an association between silica exposure and the risk for RA, relatively very little is known regarding the mechanism by which silica can lead to the development of RA, and it was not known at the time of initiation of our study whether silica had different effects in the ACPA+ *vs* ACPA- forms of RA.

1.5.3 Other environmental risk factors and effects of environmental agents on the two different RA subsets

Besides smoking and silica, there are several other environmental risk factors that have been associated with the risk of RA, but where few or no studies have been performed in Asian populations. Thus, there is a need to expand studies on environmental and life style factors in Asian populations to enable better preventive measures of RA in large parts of the world. Further, the recognition that RA may exist in two distinct groups determined by the presence of ACPA (82-84), and that genetic as well as environmental factors may act differently in these two subsets of RA, makes studies on environment and life style in RA separated by serology, an important area of investigation (see also [85,86]).

So far, apart from smoking and silica, the following risk factors have been subject to study in Caucasian populations: Alcohol, vitamin D, oral contraceptives, breast feeding, birth weight, dietary factors and socioeconomic status. These are factors that have been associated with the development of RA, taking into consideration the two subsets of RA, either by RF or ACPA [54]. Also, results from two Scandinavian case-control studies found that moderate alcohol intake was associated with reduced risk of RA (ACPA positive and well as ACPA negative RA) [59]. This finding was supported by a prospective cohort study with repeated measurement among women undergoing mammography screening in Sweden [61].
Further, occupational exposure to mineral oil was found to be associated with an increased risk for both RF and ACPA+ RA (but not negative) [63]. Obesity was associated with an increased risk of developing ACPA- RA among women [81].

1.6 GENETIC RISK FACTORS

Studies in twins with rheumatoid arthritis have shown that genetic factors substantially contribute to the development of the disease [44]. The large importance of environment has also been illustrated by studies on twins. In a study on 13 RA-discordant monozygotic twins (pairs), 12 out of 13 of the RA cases were smokers [47].

Several genes have been identified and associated with increased risk of RA. The strongest and most reproducible associations were demonstrated within the human leukocyte antigen (HLA)

region, particularly the HLA-DRB1 gene. The HLA-class II alleles are known to be the most important genetic risk factor for RA [82], and the HLA-DRB1 alleles that code a "shared epitope" are associated with a higher risk of developing RA [83], particularly ACPA+ RA [72, 84-86]. The PTPN22 gene, another important genetic risk factor was also found to be associated with the risk for RA [87-89]. A number of genome-wide association studies (GWAS) on individuals with autoantibody-positive RA of European descent have defined more than 40 RA risk alleles [90]. The genetic patterns of MHC associations among Caucasian populations were distinct between ACPA+ RA and ACPA- RA also when using the genome-wide genetic approach [85].

As stated above, most studies on genetic susceptibility for RA have been performed on Caucasians and susceptibility genes may be different in other populations. Thus, for the most important genes, HLA-DR, the frequency of SE alleles among RA patients varied significantly among ethnic groups, where *0401 and *0404 alleles are predominantly associated with RA among Caucasians [91, 92] and the *0405 allele being predominantly associated with RA in Japanese, Korean and Chinese [93-95]. Furthermore, a meta-analysis among rheumatoid arthritis patients found that SE alleles is associated with the development of erosive disease with significant differences across ethnic groups [96].

2 AIMS

2.1 GENERAL AIM

The general aim of the thesis was to investigate the influence from smoking and silica (as respiratory environmental risk factors), including their potential interaction with the most important genetic risk factor in RA, on the development of RA in the Malaysian and Swedish populations.

2.2 SPECIFIC AIMS

- To study the association between cigarette smoking and the risk of development of ACPA+RA, ACPA-RA and RA overall among the Malaysian population (MyEIRA study).
- To study the interaction between smoking and HLA-DRB1 SE alleles with regard to risk of ACPA+RA among the Malaysian population (MyEIRA study).
- To study the influence from occupational exposure to silica on the risk of developing ACPA+ RA, ACPA-RA and RA overall among men in a Caucasian population (EIRA study).
- To study the influence from occupational silica exposure on the risk for ACPA+ RA, ACPA-RA and RA overall among men in an Asian population (MyEIRA study).

3 THE HISTORY OF MyEIRA

3.1 THE RATIONALE OF THE MYEIRA STUDY

The knowledge gained so far about environmental and genetic risk factors and the risk of RA is mainly based on studies of Caucasian populations. In Caucasians smoking has been observed to be a major risk factor for the more severe form of RA, i.e. ACPA+RA, but not ACPA-RA. Smoking has further been found to interact with the most important genetic risk factor in RA, HLA-DRB1 SE, with regard to ACPA+RA. The question as to whether the same risk factors are also present in other populations with different genetic backgrounds and with different life styles and different patterns of environmental exposures remains unanswered.

In order to answer, among others, these research questions, a study was initiated with the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia, where a sister study to the Swedish EIRA study was launched in 2004 called MyEIRA. In MyEIRA the design of the original EIRA study was adjusted to local conditions and possibilities in Malaysia. The overall purpose of the MyEIRA study was to investigate the influence of environmental and lifestyle factors on the risk of developing RA in the Malaysian population. Malaysia is unique because it provides an opportunity to study three major ethnic groups in Asia and offers a good prospect of studying the influence of similar environmental and lifestyle factors across different genetic backgrounds. MyEIRA thereby allows an opportunity to assess the impact of environmental factors in different genetic contexts and may provide further insight into gene-environment interaction in RA.

4 METHODS AND MATERIALS

4.1 THE MALAYSIAN EPIDEMIOLOGICAL INVESTIGATION OF RHEUMATOID ARTHRITIS (MYEIRA) (PAPER I, II AND IV)

The MyEIRA study, is a sister study to the Swedish EIRA study. It is a case-control study where the subjects were recruited between August 2005 and December 2009 throughout Peninsular Malaysia. The study consists of 1056 cases and 1416 controls (907 female cases and 1203 female controls, 149 male cases and 213 male controls), with a participation proportion of 92% amongst cases, 76% for controls.

The incident cases were diagnosed by rheumatologists according to the 1987 American College of Rheumatology (ACR) criteria for RA [25] at the participating centres. Nine rheumatology centres of the Ministry of Health Malaysia contributed with cases. Eight centres were located on the west coast of Peninsular Malaysia and one was located on the east coast.

For each case, at least one control was selected randomly, taking into consideration age, gender and place of residence of each respective case. In the MyEIRA study two forms of control groups were selected: the population based controls and hospital-based controls. In the first two years of the study period, controls were selected from the hospital where each case was recruited. They were carefully chosen from among people accompanying patients, hospital staff and patients without an inflammatory disease. Later, we randomly selected population controls. With the help of public health nurses and based on cases' addresses, we visited the areas and personally got to know the neighbourhood. We explained to a potential control about the study and encouraged them to voluntarily participate in the study. Withdrawal from the study at any time was allowed by informing the study centre. If an eligible control decided not to participate, another control was recruited based on the same principles. Written consent was obtained from each case and control. Ethical approval was obtained from the Medical Research and Ethics Committee, Ministry of Health Malaysia.

Information regarding a wide spectrum of factors, such as socio-economic background, previous medical history, heredity, reproductive data, body weight and height, lifestyle factors, occupational exposures and psychosocial circumstances were gathered through interviews according to a questionnaire. Identical questions were used for cases and controls. The questionnaire used was based on the questions used in the Swedish EIRA study some of which were adjusted to suit the Malaysian culture. As stated, all information was collected by means of a face-to-face interview by well-trained interviewers. Unanswered or incomplete questionnaires were completed by phone or mail by study secretaries. Each case and control provided blood samples for serum and DNA analyses. The details of this study can be found in Paper I.

4.2 THE SWEDISH EPIDEMIOLOGICAL INVESTIGATION OF RHEUMATOID ARTHRITIS (EIRA) STUDY (PAPER III)

The Swedish EIRA study is the world's largest population based case-control study on RA with concomitant information on both environmental/lifestyle factors and genetics. Data used in this thesis were generated between May 1996 and May 2006 with a total of 1997 cases and 2252 controls (of which 577 male cases and 659 male controls were used in Paper III). The participation proportions were 95% amongst cases and 81% amongst controls. All incident cases (85% were diagnosed within a 12 months from onset of first symptom) were diagnosed by rheumatologists

according to the 1987 ACR criteria for RA. Cases were from rheumatology clinics in Stockholm and parts of southern Sweden.

For each case, a control was selected randomly from the population using the Population Register (which is continuously updated), taking into consideration age, sex and place of residence of the case. Information on environmental exposures was collected using an identical questionnaire. The cases received their questionnaires right after the diagnosis and were expected to answer it at home. Questionnaires for controls were mailed to them. Completed questionnaires from both cases and controls were mailed to the study centre. Incomplete information was completed through phone calls and mail by well-trained secretaries.

4.3 ENVIRONMENTAL EXPOSURES AND THE RISK FOR RA

In this thesis, we were focusing on two main environmental exposures related to the lungs: smoking and silica exposure.

4.3.1 Smoking

For both EIRA studies, the information about smoking was determined in a similar manner. Five questions were asked about smoking habits as follows: (1) Have you ever smoked? (2) If you do not smoke, have you previously smoked? (3) If you have previously smoked, which year did you stop smoking? (4) If you smoke or previously have smoked, when did you start to smoke regularly? (5) How much do you smoke, or did you smoke before you stopped, on average per day? Information about other types of tobacco smoking was also obtained.

In estimating the effect of smoking on the risk of developing RA, we only considered smoking exposure before or during the index year. Index year was determined as the year when the first symptoms of RA occurred. The same index year was assigned to the corresponding control. The smoking habits were classified into four categories: never smoker, current smoker, former smoker and ever-smoker. We defined never smokers as those who had never smoked before or during the index year, while current smokers were those who were regularly smoking during the index year, former smokers as those who stopped smoking before the index year and ever-smokers were those who were either current or former smokers.

4.3.2 Silica

In both EIRA studies, silica exposure was determined as occupational exposure to rock drilling or stone crushing or stone dust. Persons who were exposed to work-related stone dust, rock-drilling or stone crushing up to the index year were defined as exposed to silica.

4.4 SEROLOGICAL ANALYSIS AND GENOTYPING

In the MyEIRA study, all study participants provided a blood sample. ACPA was measured using an anti-cyclic citrullinated peptide (anti-CCP) second-generation enzyme-linked immunosorbent assay (ELISA) kit (Immunoscan RA Mark 2, Euro-Diagnostica, Malmö, Sweden) for all samples. A presence (marked as positive) of ACPA was considered if the serum antibody level exceeded 25 U/ml. High resolution of HLA-DRB1 genotyping was performed for shared-epitope (SE) alleles by

the PCR-SSO method described elsewhere [97]. The HLA-DRB1 shared-epitope group was defined by HLA-DRB1*0101, *0102, *0107, *0401, *0404, *0405, *0408, *0410, *1001, and *1003. Only a small fraction 46 of 1054 (4.4%) of the cases carried two copies of HLA-DRB1 SE alleles. Hence, we decided to limit the analysis by investigating the influence of any SE allele (defined as the presence of either one or two copies of SE alleles) on the risk of RA.

The methods and definition of the presence of ACPA used in the Swedish EIRA study were used accordingly in the MyEIRA study [72]. Whereas HLA-DRB1 genotypes were made using the sequence-specific, primer-polymerase chain reaction method (DR low- resolution kit; Olerup SSP, Saltsjobaden, Sweden) as described elsewhere [98].

4.5 POTENTIAL CONFOUNDERS

In Papers I-IV, age, gender and residential area were matched according to design considerations. Other potential confounding factors considered were formal education, a marker for socio-economic status, and ethnicity (in MyEIRA). Adjustment for body mass index (BMI) was also made but since its influence was minor it was not retained in the final analysis.

Except for age, residential area and social class, joint injury and physical workload were considered as potential confounders in Paper III.

4.6 STATISTICAL ANALYSIS

The analyses were performed using conditional logistic regression in estimating odds ratio together with 95% confidence interval (95% CI) in Paper I and II. By contrast in Paper III and IV, we used unconditional logistic regression. In Paper IV, we also performed matched analyses but we only present results from the unmatched analyses, since the results were in close agreement with the matched analyses but had higher precision. ORs were calculated for developing ACPA+ RA, ACPA- RA and RA overall.

Interaction between factors (Papers II-IV) was defined by departure from additivity of effects. Presence of interaction was evaluated by calculating the attributable proportion due to interaction (AP) together with the 95% CI. AP is the proportion of the incidence among persons exposed to two interacting factors that is attributable to the interaction per se [99].

5 RESULTS

The results are mainly based on the MyEIRA study, and are presented in Papers I, II and IV. In Paper III, the results were entirely based on the Swedish EIRA study. In the MyEIRA study, a total of 1056 primarily incident cases and 1416 controls were analysed, giving a participation proportion of 92% among cases and 76% among controls. In the Swedish EIRA study, the participation proportion was observed to be 95% among cases and 81% among controls (Paper III).

5.1 PAPER I - CIGARETTE SMOKING AND THE RISK OF ACPA+ RA IN THE MYEIRA STUDY

Ever-smokers had an increased risk of developing ACPA+ RA compared with never-smokers (OR=4.1, 95% CI 1.9-9.2). The increased risk for ACPA+RA was observed among both men and women (men OR=5.2, 95%CI 1.7-16.1, women OR=3.6, 95%CI 0.7-18.4). No increased risk for ACPA- RA was observed among ever-smokers (OR=0.7, 95%CI 0.3-2.0). In order to study the dose-response relationship between cigarette smoking and RA risk, the cumulative dose of smoking expressed by pack-years was calculated. A statistically significant dose-response relationship between cumulative dose of smoking and the risk of ACPA+ RA was observed (*p*-trend <0.0001) (Table 1).

Table 1 Odds ratios (OR) with a 95% confidence interval (95% CI) of developing ACPA+ RA, ACPA- RA and RA overall for ever-smokers compared to never-smokers together with the *p*-value for trend test of cumulative dose of smoking in relation to risk of ACPA+RA.

	RA overall			**ACPA+ RA**			**ACPA- RA**		
	Ca/Co[a]	OR[b]	95% CI[b]	Ca/Co	OR[b]	95% CI[b]	Ca/Co	OR[b]	95% CI[b]
Never-smokers	927/1260	1.0	Ref	577/1260	1.0	Ref	350/1260	1.0	Ref
Ever-smokers[a]	79/65	2.2	1.2 - 3.9	64/65	4.1	1.9 - 9.2	15/65	0.7	0.3 - 2.0
1-19 pack-years[c]	35/29	2.7	1.2 - 6.4	26/29	3.3	1.1 - 9.8	9/29	1.9	0.5 - 8.0
≥20 pack-years	33/22	2.3	1.0 - 5.5	28/22	5.2	1.6 - 17.6	5/22	0.4	0.1 - 2.3
p-value						<0.0001[d]			

[a] A total of 91 controls and 50 cases lacked smoking information
[b] OR and 95% CI with additional adjustment for formal education and ethnicity
[c] A total of 13 controls and 11 cases lacked information on pack-years.
[d] *p*-value for trend regarding OR for pack-years regarding ACPA+ RA
Ref: Reference group
Ca/Co number of cases and controls

5.2 PAPER II - INTERACTION BETWEEN SMOKING AND HLA-DRB1 SE REGARDING RISK OF DEVELOPING ACPA+ RA; MYEIRA STUDY

Ever-smokers with any SE alleles were observed to have a substantially increased risk of ACPA+ RA (OR $_{\text{ever-smoking-any SE}}$= 25.6, 95%CI 10.4-63.4). The observed risk exceeded the sum of individual effects associated with smoking and SE (OR $_{\text{ever-smoking- no SE}}$=3.1, 95%CI 1.7-5.7 and OR $_{\text{never-smoking – any SE}}$=4.4, 95%CI 3.5-5-6), and this indicates the presence of interaction on the additive scale. The attributable proportion due to interaction was 70% (95%CI 0.5-1.0), which indicates that around 70% of the ACPA+ RA cases among those that had ever smoked and carried SE alleles was attributed to the interaction between smoking and SE. The most common allele in Asian populations, the HLA-DRB1*0405, in combination with smoking was associated with an increased risk of ACPA+ RA (OR=12.9, 95%CI 4.7-35.3), and revealed a sign of interaction although it was statistically insignificant (AP=0.4, 95%CI -0.1-0.9) (Table 2).

Table 2 Odds ratios for developing ACPA+ RA and ACPA- RA in subjects with different combinations of smoking and presence of any SE allele and HLA-DRB1*04:05 SE alleles, compared with never-smokers without SE alleles.

	No SE			**Any SE**			**HLA-DRB1*04:05 SE alleles**		
	Ca/Co	OR*	95% CI	Ca/Co	OR*	95% CI	Ca/Co	OR*	95% CI
ACPA+ RA									
Never-smokers	266/986	1.0	Ref	264/202	4.4	3.5 - 5.6	102/71	5.4	3.8 - 7.5
Ever-smokers	30/52	3.1	1.7 - 5.7	33/8	25.6	10.4 - 63.4	16/7	12.9	4.7 - 35.3
AP					0.7	0.5 - 1.0		0.4	-0.1 - 0.9
ACPA- RA									
Never-smokers	254/986	1.0	Ref	65/202	1.1	0.7 - 1.7	20/71	1.1	0.7 - 1.8
Ever-smokers	10/52	0.6	0.2 - 2.0	3/8	0.8	0.1 - 4.8	1/7	0.6	0.1 - 4.8

*OR adjusted for age, gender, residential place, formal education and ethnicity
HLA-DRB1*04:05 group was defined by the presence of either one or two copies of HLA-DRB1*04:05 allele (individuals with first allele of HLA-DRB1*04:05 and second allele of non-HLA-DRB1*04:05 SE were excluded)
Ref: Reference group
Ca/Co number of cases and controls

5.3 PAPER III - SILICA EXPOSURE, SMOKING AND THE RISK OF ACPA+ RA IN THE SWEDISH EIRA STUDY

Moderate increased risk of ACPA+ RA was observed among men exposed to silica (OR=1.67, 95%CI 1.13 - 2.48) compared to subjects never exposed to silica. On the other hand, no increased risk was observed for developing ACPA- RA (OR=0.98, 95%CI 0.57 - 1.66) (Table 3). Exposure to rock drilling was found to lead to a somewhat more markedly increased risk of ACPA+ RA (OR=2.34, 95%CI 1.17 - 4.68). Silica-exposed male current smokers were observed to have a specially high risk of ACPA+ RA (OR=7.36, 95%CI 3.31 - 16.38), in which about 60 per cent of the risk for developing ACPA+ RA was attributed to the interaction between silica and current smoking (AP=0.60, 95%CI 0.26 - 0.95) (Table 4). There was a sign of interaction between silica exposure and any HLA-DRB1 SE allele, however the estimated confidence interval was broad (AP=0.18, 95%CI -0.33 - 0.68).

Table 3 Odds ratio (OR) with 95% confidence interval (CI) of developing ACPA+ RA and ACPA- RA among men exposed to silica, compared with men unexposed to silica.

	ACPA+ RA			**ACPA- RA**		
	Ca/Co	OR*	95% CI	Ca/Co	OR*	95% CI
Unexposed to silica	275/578	1.0	Ref	183/578	1.0	Ref
Silica exposed	54/69	1.67	1.13 - 2.48	20/69	0.98	0.57 - 1.66

Ca/Co number of cases and controls

Ref: Reference group

*OR adjusted for age and residential area.

Table 4 Odds ratio (OR) with 95% confidence interval (CI) of developing ACPA+ RA and ACPA- RA among men with different combinations of silica exposure status and cigarette smoking, compared with men unexposed to silica and never-smokers.

	Unexposed to Silica			**Exposed to Silica**		
	Ca/Co	OR*	95% CI	Ca/Co	OR*	95% CI
Never-smokers						
ACPA+ RA	57/193	1	Ref	6/17	1.15	0.42 - 3.15
ACPA- RA	54/193	1	Ref	4/17	0.85	0.26 - 2.75
Ever-smokers						
ACPA+ RA	176/284	2.53	1.72 - 3.72	38/41	4.08	2.31 - 7.21
ACPA- RA	101/284	1.23	0.83 - 1.84	13/41	1.16	0.56 - 2.39
Ex-smokers						
ACPA+ RA	99/174	2.52	1.63 - 3.89	17/28	2.84	1.39 - 5.84
ACPA- RA	57/174	1.03	0.65 - 1.62	9/28	1.05	0.45 - 2.46
Current smokers						
ACPA+ RA	77/110	2.78	1.77 - 4.38	21/13	7.36	3.31 - 16.38
ACPA- RA	44/110	1.43	0.88 - 2.34	4/13	1.16	0.35 - 3.87
AP					0.60	0.26 - 0.95

Ca/Co number of cases and controls

*OR adjusted for age and residential area.

Ref: Reference group

AP: Attributable proportion due to interaction (between current smokers and silica exposed)

5.4 PAPER IV - SILICA EXPOSURE, SMOKING AND THE RISK OF ACPA+ RA IN THE MYEIRA STUDY

An increased risk of ACPA+ RA was observed among men exposed to silica (OR=2.4, 95%CI 1.0 - 5.6) compared to men not exposed to silica. No increased risk was observed for ACPA- RA (Table 5). We observed that silica-exposed ever-smokers had an especially high risk of developing ACPA+ RA compared to never-smokers not exposed to silica (OR=7.5 95% CI 2.3 - 24.2). A synergistic interaction between smoking and silica was present with regard to development of ACPA+ RA (AP=0.5 95%CI -0.5 - 1.5) (Table 6).

Table 5 Odds ratio (OR) with 95% confidence interval (CI) of developing ACPA+ RA and ACPA- RA among men exposed to silica, compared with men unexposed to silica.

	Exposed to Silica	**Unexposed to Silica**		
RA by subtype	**Ca/Co**	**Ca/Co**	**OR***	**95% CI**
ACPA+ RA	12/12	82/194	2.4	1.0 - 5.6
ACPA- RA	2/12	41/194	0.9	0.2 - 4.5
RA overall	14/12	123/194	2.0	0.9 - 4.6

Ca/Co Number of exposed cases/ number of exposed controls
OR (95% CI): odds ratio and 95% confidence interval
OR* adjusted for age and residential area

Table 6 Odds ratio (OR) with 95% confidence interval (CI) of developing ACPA+ RA and ACPA- RA among men with different combinations of silica exposure and smoking habits, compared with men never-smokers unexposed to silica.

	No Silica			**Silica**		
	Ca/Co	**OR***	**95% CI**	**Ca/Co**	**OR***	**95% CI**
Never-smoker	31/102	1.0	Ref	0/2	-	-
Ever-smoker	41/52	2.7	1.5 - 4.9	11/5	7.5	2.3 - 24.2
AP					0.5[a]	-0.5 - 1. 5

Ca/Co Number of exposed cases/ number of exposed controls
OR (95% CI): odds ratio and 95% confidence interval
OR* adjusted for age and residential area
Ref: Reference groupAP: Attributable proportion due to interaction
[a] One case has been added in the Never-smoker/Silica exposed group to be able to calculate the AP (will lead to underestimation of the AP)

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATION

6.1.1 Study design

Data in this thesis were based on two population based case-control studies: the Swedish EIRA study and the Malaysian EIRA study, comprising the general population aged between 17-80 years. In the Swedish EIRA study, the recruitment of subjects took place between May 1996 and May 2006. Subjects were selected from a geographically defined part of Sweden.
The subjects in the MyEIRA study were recruited between August 2005 and December 2009. In this study, the cases were generated from all rheumatology centres under the Ministry of Health Malaysia throughout the Peninsular Malaysia.
The case-control design in both EIRA studies provides an efficient way to study the association between different exposures (in our case, environmental exposures/ lifestyle factors and the genetic factors) and disease (rheumatoid arthritis). It could be conceptualised as a more efficient form in relation to that of a cohort design. By careful sampling of controls from the population that gave rise to the cases, the case-control odds ratio approximately estimates the rate ratio that would be obtained in a cohort design. Furthermore, case-control design is more cost effective and usually requires shorter time than a cohort design.

For each case, a control was recruited and matched according to the age, sex and residential area of the case. As RA is known to be more common in certain age categories, females and geographical areas, matching on these variables could enhance the efficiency in the stratified analyses. On the other hand, matching does not allow us to examine the association between the matching factors and the disease understudy.

The mode of gathering information from the study subjects displays a major difference between the Swedish EIRA and the MyEIRA. In the Swedish EIRA study, questionnaires were handed to the cases immediately after they were diagnosed with RA, and the controls received their questionnaires by mail. Both cases and controls in the Swedish EIRA study were expected to answer the questionnaires by themselves at home. By contrast, in the MyEIRA study, this method was not feasible. As a matter of fact, we conducted face-to-face interviews to obtain information on lifestyle and environmental exposures from both cases and controls.

Furthermore, in the beginning of the two years of the MyEIRA study, we recruited hospital controls among those people who accompanied patients, patients with other conditions than autoimmune or joint diseases and hospital staff. This might not be an optimal control group however. In order to utilize this group, we performed separate analyses for each study to see whether this group would alter the result somehow. Nevertheless, we found that these two groups of controls were comparable and we decided to retain them in the final analyses to increase the power of study.

6.1.2 Selection bias

A high participation proportion among cases and controls in both EIRA studies (95% for cases and 81% for controls in the Swedish EIRA study; 92% for cases and 76% for controls in the MyEIRA study) should limit the potential bias due to selection. In the Malaysian EIRA study, the smoking prevalence among controls differed to some extent from the latest nation-wide survey, the National Health and Morbidity Survey III [100]. In this survey, the prevalence of ever-smoking was 58%

among men and 2.5% among women, aged between 13 to more than 80 years. Thus, we cannot be certain that controls did not somehow underreport their smoking habits in our study (men=36% and women=1%) and as a consequence to some extent overestimated the OR associated with smoking and the risk of RA. This however, will not affect our main finding that smoking mainly increased the risk for ACPA+ RA and not ACPA- RA, as it is unlikely that cases of different subtypes recalled their smoking habits differently.

6.1.3 Misclassification of exposures

In a case-control design study, information on exposure usually is collected retrospectively, and recall bias may thus be a concern. Differential misclassification or recall bias occurs when cases recall their exposures differently from the controls. This phenomenon is common among prevalent cases. However, in both the Swedish and Malaysian EIRA studies, we recruited cases with a short duration since disease onset in an attempt to overcome this matter. In the Swedish EIRA study, cases were given the questionnaire right after the diagnosis of RA, where about 85% of the cases had a duration of 12 months or less between the estimated disease onset and inclusion in the study. Whereas, in the MyEIRA study, RA cases were selected by rheumatologists based upon their medical records and the median duration of disease among the cases was one year with an interquartile range of two years. In both scenarios, it was unlikely that cases recalled their exposures, in our context their smoking habits and silica exposure, very differently from the controls. Our results in both EIRA studies with regards to smoking and silica exposure, demonstrated an increased risk of ACPA+ RA but not ACPA- RA, which also speak against a high extent of recall bias, since it is highly unlikely that ACPA+ RA cases report their exposures very differently than ACPA- RA cases.

6.1.4 Misclassification of disease

Both EIRA studies utilised identical diagnostic and classification criteria: the 1987 ACR criteria for RA [25]. These criteria are easy to use and have clear definitions, although sometimes they have a limitation on early RA cases or less severe RA, in which there are common features for ACPA- RA. Nevertheless, using the same case definition could ease the comparison between EIRA studies as well as in combining data for future analyses.

Another potential source of bias in both EIRA studies is the fact that some cases may have not been included in the studies. Fail to report diagnosed cases of RA, in either study, is probably mainly due to administrative reasons, such as, high burden of work, change of personnel (that are not informed about the study), etc., i.e. for reasons that were not related to smoking and/or silica exposure. We thus believe that bias due to misclassification of disease does not constitute a major bias.

6.1.5 Confounding

In both EIRA studies, age, sex and residential area were matched according to design consideration. Other potential confounders, such as occupation (in Swedish EIRA) and formal education (in MyEIRA), both markers for social class, were also taken into consideration and adjusted for in the analyses (Paper I and II). In Paper III and IV, this factor marginally changed the estimates, and therefore was not retained in the final analyses. Joint injury and physical workload were considered as potential confounders in Paper III but adjustment for them have little influence on the estimates

and yet were not retained in the final analyses. In MyEIRA, ethnicity was an important confounder as a marker of genetic background, it was adjusted for in Papers I and II but not in Paper III, as the influence in Paper III was so little. Adjustment for BMI was also made (Paper 1) but this had only minor influence on ORs associated with smoking, therefore was not retained in the final analyses.

6.2 COMMENTS ON PRESENT RESULTS AND PREVIOUS STUDIES

Overall, our results demonstrated that modifiable lung exposures, here smoking and occupational silica exposure, are associated with an increased risk of developing ACPA positive rheumatoid arthritis, but not ACPA negative in an Asian population.

In the first study among the Malaysian population (Paper I), intriguingly, we found that smoking was significantly associated with an increased risk of ACPA+ RA. These results are consistent with a large number of results from Caucasian populations and from countries where the frequency of smoking is considerably higher than in Malaysia. Although the prevalence of ever-smoking among controls differed to some extent from the latest national wide morbidity survey in Malaysia, the National Health Morbidity Survey III, it would not affect the main finding: that smoking influences the risk of developing ACPA+ RA to a much higher extent than it influences the risk of ACPA- RA [100]. Interestingly, an increased risk was observed for both men and women, yet the risk was inconclusive for women due to a low number of exposed individuals in this group. Furthermore, we also observed a significant dose-response relationship between cumulative dose of smoking and risk of ACPA+ RA.

The knowledge gained from this study strengthens the concept that smoking is a risk factor for ACPA+ RA (in several different populations and that this risk is thus not limited to Caucasians, where most studies have been performed so far [7, 68, 69, 73, 101]).

We further investigated the influence of the most prominent genetic risk factor, the HLA-DRB1 SE alleles, together with the presence of an environmental risk factor, smoking, in the development of rheumatoid arthritis in the Malaysian population. Our data demonstrated that the combined effect of HLA-DRB1 SE alleles and smoking increased the risk of ACPA+ RA more than the sum of individual effects, i.e. a synergistic interaction was present. No increased risk was observed for ACPA- RA, neither with presence of SE alleles, smoking nor their combination. This is accordance with other studies, both among Swedish, Dutch and Danish populations [72, 102, 103], but only partly in accordance with a smaller Korean study where the influence of smoking on risk for RA was reported for both ACPA+ and ACPA- disease [71]. We also demonstrated that the most common SE allele for Asians, the HLA-DRB1*0405, was associated with an increased risk of ACPA+ RA, something that has not been demonstrated before. This allele also displayed a sign of interaction with smoking, however it was statistically insignificant. Our sister study, the Swedish EIRA study has previously demonstrated that smokers with HLA-DRB1*0401 and/or *0404 allele (the most common allele among Caucasians) are at a particular high risk of developing ACPA+ RA, and that a significant interaction between those risk factors exits (AP=0.4 95%CI 0.2 - 0.6) [92]. Our findings provide additional knowledge for understanding the pathogenesis of RA in Asian populations.

After studying the effect of smoking and the influence of susceptibility genes in relation to the risk of developing RA in the Malaysian population, we further investigated the influence of another respirable exposure, silica, on the risk of RA subtypes defined by presence of ACPA. We began by observing the associations in the Swedish EIRA data and then further investigated whether the same associations were as also present in a non-Caucasian population: the Malaysian population. Our results from the Swedish EIRA study indicated that silica-exposed subjects have a moderately

increased risk of ACPA+ RA compared to subjects unexposed to silica. No increased risk was observed for ACPA- RA. We also observed that smoking had an influence among silica-exposed subjects: current smokers among silica-exposed subjects had a particular high risk of ACPA+ RA. It was further noted that there was a statistically significant interaction between current smoking and silica with regard to risk or ACPA+ RA. This is the first study to describe the association between silica exposure and the risk of RA by subtype defined by presence of ACPA. We believe that the knowledge from this study could serve as a valuable contribution towards understanding the pathogenesis of RA as these data further strengthens the hypothesis of the lung as an important organ involved in the aetiology of some cases of RA.

In the Malaysian EIRA study, despite the small number of subjects exposed to silica, we demonstrated a similar finding to the Swedish EIRA study: i.e. that exposure to silica increases the risk of ACPA+ RA and that silica-exposed smokers are at a particularly high risk of developing ACPA+ RA. There was also an indication of synergistic interaction between silica exposure and smoking although this was not statistically significant. Regardless of ethnicity, the findings from these studies demonstrate that lung exposures may play an important role in pathogenesis of RA. Our findings suggest that smoking prevention as well as reduction of silica exposure could decrease the burden of RA in many parts of the world.

6.3 FINAL REMARKS AND FUTURE RESEARCH

Understanding the aetiology of RA is still a large puzzle that needs to be solved. Epidemiological studies on environmental exposure as well as those done on environmental factors in combination with genetic risk factors have added some valuable knowledge in understanding the pathogenesis of RA. In this thesis, we have demonstrated that modifiable lung exposures, here smoking and occupational silica exposure, are associated with an increased risk of ACPA+ RA but not ACPA- RA, regardless of ethnic differences.

Smoking has been found to be associated with an increased risk of developing ACPA+ RA and RF+ RA in the Swedish EIRA study as well as in other studies among Caucasians [53, 68, 69, 74, 104]. The risk of ACPA+ RA was observed to be even higher among smokers with the most susceptible genetic risk factor, leading to a strong gene-environment interaction between smoking and the HLA-DRB1 SE alleles [72, 73, 102, 103]. In the MyEIRA study population, with a different genetic constitution than Caucasians, we found the similar result, i.e. that ever-smokers had a greater risk for developing ACPA+ RA - but not ACPA- RA - compared with non-smokers. This finding indicates that mechanisms behind the association between smoking and RA may be similar in Malaysian and Caucasian populations. It also provides an interesting basis for the further study of the influence of HLA-DRB1 SE alleles among smokers, particularly in relation to risk of ACPA+ RA in the Malaysian population.
An important message from our study is that smoking is a major preventable risk factor for RA also in Asian populations. We hope that this information will help policy makers in fighting against the global tobacco epidemic. According to the WHO report on this epidemic from 2011, it kills nearly 6 million people every year and by 2030, more than 8 million people could die from it, with 80% of deaths occurring in low-and middle-income countries [105]. According to the findings in this thesis public health initiatives aimed at decreasing smoking probably will help in preventing RA development in Asian populations.
The synergistic interaction between smoking and silica in both Caucasian and Asian populations strengthens the hypothesis that lung exposures may play an important role in the aetiology of ACPA+ RA. These findings also suggest that respirable environmental risk factors should be taken into consideration when studying the aetiology of RA as well as in efforts aimed at preventing this disease.

Future research

Interestingly, from a public heath point of view, our findings from MyEIRA on the importance of exposure to smoking and silica mainly concerns men, since these exposures are more prevalent among men than among women. We believe that knowledge from this thesis could be useful in future studies looking for associations between other respirable exposures, either from leisure and/or occupational activities, with regards to the risk of ACPA+ RA, including their interaction with susceptible genes, perhaps with a special focus on women.

7 CONCLUSIONS

- Smoking was associated with an increased risk of ACPA+ RA but not ACPA- RA in the Malaysian population. The risk of ACPA+RA was influenced by the cumulative dose of smoking in a dose-dependent manner. The results showed that smoking is also an important environmental risk factor in an Asian population.

- A gene-environment interaction was observed between HLA-DRB1 SE alleles and smoking with regard to an increased risk of developing ACPA+ RA in an Asian population. Also for the HLA-DRB1*0405 allele, the common allele among Asian populations, there were signs for an interaction with smoking. The data illustrates the importance of taking gene-environment interaction into account when conducting studies on complex diseases.

- Among males, occupational exposure to silica was associated with an increased risk of ACPA+ RA but not ACPA- RA in a Caucasian population. An interaction was observed between current cigarette smoking and silica exposure with regard to risk of developing ACPA+ RA, suggesting that different respirable exposures may interact in the aetiology of ACPA+ RA.

- Also in an Asian population, silica-exposed males were observed to have an increased risk of developing ACPA+ RA, but not ACPA- RA, and there were signs of an interaction between silica exposure and smoking with regard to ACPA+ RA, a finding that strengthens the hypothesis that this lung exposure may play a role in the aetiology of ACPA+ RA, regardless of ethnicity.

8 SAMMANFATTNING PÅ SVENSKA

Reumatoid artrit (RA) är en kronisk, inflammatorisk autoimmun sjukdom som ofta innebär leddestruktion. Kunskap om etiologin till RA har främst genererats bland kaukasiska populationer. Mindre är känt om andra populationer med annan genetisk bakgrund och livsstil. Syftet med denna avhandling var att bidra till en bättre kunskap om etiologin till RA genom att studera sambandet mellan RA och luftvägsexponeringar (i detta arbete rökning och yrkesmässig exponering för kvartsdamm), i en asiatisk befolkning. Vi studerade risken att utveckla RA samt de olika subtyperna av sjukdomen, som definieras av närvaro eller frånvaro av antikroppar mot citrullinerade proteinantigener (ACPA+ RA och ACPA- RA).

Denna avhandling baseras huvudsakligen på den malaysiska Epidemiologiska undersökningen av Reumatoid Artrit (MyEIRA) (delarbete I, II och IV). MyEIRA är en befolkningsbaserad fall-kontrollstudie med omfattande information om livsstil och yrkesmässig exponering. Fall och kontroller lämnade också blodprov för serologisk och genetisk analys. I delarbete III användes data från den svenska befolkningsbaserade fall-kontrollstudien EIRA. Information om de miljömässiga exponeringarna samlades in via frågeformulär. Fall och kontroller lämnade dessutom blodprov för genetisk och serologisk analys.

Våra resultat i MyEIRA studie visar att rökare hade en ökad risk att utveckla ACPA+ RA jämfört med dem som aldrig rökt, men ingen ökad risk för ACPA- RA observerades. Ett signifikant dos-responssamband mellan kumulativ dos av rökning (definierat som pack-years) och risken för ACPA+ RA observerades. En signifikant interaktion mellan rökning och HLA-DRB1 shared epitope (SE) alleler observerades för risken för att utveckla ACPA+ RA, men inte för ACPA-RA. Vi fann också att den vanligaste allelen i den asiatiska befolkningen, HLA-DRB1 * 0405, också visade tecken på interaktion med rökning vad gällde en ökad risk för ACPA + RA.

Vi studerade vidare yrkesmässig exponering för kvartsdamm, d v s en annan luftvägsexponering, och risken att utveckla RA i den svenska EIRA studien. Män exponerade för kvartsdamm hade en måttligt ökad risk för ACPA + RA men inte ACPA-RA, jämfört med män som aldrig exponerats för kvartsdamm. En signifikant interaktion mellan kvartsdamm och nuvarande rökning observerades i risken för ACPA + RA. Vi fann liknande resultat i MyEIRA studien, där yrkesmässig exponering av kvartsdamm var associerat med en ökad risk för ACPA+ RA, men inte för ACPA- RA. Det fanns även indikationer på interaktion mellan kvartsdamm och rökning för risken för ACPA+ RA, även om ett litet antal exponerade hindrade fasta slutsatser.

Sammanfattningsvis visar denna studie att luftvägsexponeringar är starkt förknippade med risk för RA i Malaysia. Resultaten bör ha betydelse för arbetet med att förebygga RA i denna stora del av världen, liksom för ytterligare jämförande studier som syftar till att förstå etiologin av RA i olika befolkningar.

9 APPENDIX

Questions on smoking:

1. Have you ever smoked? ☐ Yes, regularly ☐ Sometimes, for example at parties ☐ No

2. If you do not smoke, have you smoked in the past? ☐ Yes, regularly ☐ Sometimes, for example at parties ☐ No

3. If you smoked in the past, in which year did you quit smoking? ☐

4. If you smoke or used to smoke, when did you start smoking regularly? ☐

5. How much do you smoke or did you smoke before you quit, as a daily average?

   a. Cigarette ☐
   b. Cigarillos ☐
   c. Cigars ☐
   d. Pipe tobacco ☐

6. Do you use 'snus' (oral smokeless tobacco)? ☐ Yes If YES, how many days does 1 box last you? ☐

7. If you do not use 'snus', have you used 'snus' in the past? ☐ Yes ☐ No

8. Are you a passive smoker? ☐ Yes ☐ No

Questions on silica exposure:

	Yes	From-to-year:	No of time/week	No
19. Drilling	☐			☐

	Yes	From-to-year:	No of time/week	No
20. Stone-crushing	☐			☐
21. Stone dust	☐			☐

10 ACKNOWLEDGEMENTS

This work was performed at the Unit of Cardiovascular Epidemiology, Institute of Environmental Medicine (IMM), Karolinska Institutet, Stockholm, Sweden, with financial support from the Ministry of Health, Malaysia: MRG 7/2005, JPP-IMR 07-046, JPP-IMR 08-012, and JPP-IMR 08-006, and from the Swedish Medical Research Council (DNR 348-2009-6468) and Swedish Combine program. I am very grateful to the Malaysian Public Civil Department for providing me the scholarship and to Professor Lars Klareskog for providing me with additional funding during my PhD studies in Sweden. I would also like to thank the Ministry of Health Malaysia and the Institute for Medical Research (IMR) for allowing me to further my education in Sweden.

To my Swedish supervisors:
I would like to express my sincere respect and deepest gratitude to my main supervisor, Professor Lars Alfredsson for allowing me to become a PhD student and guiding me from the beginning to the final level of my study. Your trust and constant encouragement have made it possible for me to continue my research. It was such an honour to be given an opportunity to work with the EIRA study, a world class research group.

I would also like to sincerely thank my co-supervisor, Assistant Professor Camilla Bengtsson for endless encouragement, support and patience, interesting discussions and for believing in me. Your contribution to my studies was extremely helpful!

I would like to express my sincere gratitude to Professor Lars Klareskog for shrewd insights, and valued comments and suggestions, which have been of such great benefit for improvement of my work and in writing the manuscripts.

Further, I give my special thanks to Associate Professor Per T Larsson for your care and support from the very first day I set my foot in the MyEIRA project, to the final level of my study. Your dedication and encouragement are invaluable.

To my Malaysian supervisors:
I wish to express my warmest gratitude to Dr Shahnaz Murad, the principal investigator of MyEIRA study and also the Director of Institute for Medical Research (IMR), Kuala Lumpur, Malaysia for believing in me in working with the MyEIRA study as well as for all the support given for my PhD studies. Without her this project would not have been possible.

I would like to express my very great appreciation to Dr Amal Nasir Mustafa, Dr Nor Asiah Muhamad, Dr Nor Aini Abdullah for their valuable, constructive suggestions and endless support especially during the planning and development of the MyEIRA study as well as throughout my PhD studies. I will forever indebted to all of you.

My special thanks are extended to my external mentor, Normi Mustapha, for continuous support and encouragement, for pleasant discussions and for valuable advice.

I would also like to thank Too Chun Lai for the time we had together especially during the data collection, the first visit to Sweden and all cooperation and support given from the beginning of MyEIRA study until now.

I am particularly grateful for the support from the rheumatologists who actively participated in the MyEIRA study: Dr Heselynn Hussein, Dr Nor Shuhaila Shahril, Dr M Eashwary (Putrajaya Hospital, Putrajaya),Dr Wahinuddin Sulaiman (Tengku Bainun Hospital, Ipoh, Perak), Dr Muhaini

Othman (Serdang Hospital, Selangor), Dr Azmillah Rosman (Selayang Hospital, Selangor), Dr Suk Chyn Gun (Seremban Hospital, Negeri Sembilan), Dr Mohd Shahrir (Universiti Kebangsaan Malaysia Hospital), Dr Ing Soo Lau (Sultanah Aminah Hospital, Johor) and Dr Ainon Mokhtar (Tengku Ampuan Afzan Hospital, Pahang). Your contribution was invaluable.

I wish to thank various other people for their contribution to this study; Dr Hanjeet Kaur, Dr S Kasthoori, Dr Yuslina Mat Yusoff, Kee Chee Cheong, Mohd Hadzrik Nor Osman, Norziyana Idris, Laily Murat Saffie, Fadzilah Hana Mahpot, Fazilah Hanim Kornain, Mohd Arif Abd Aziz, Nor Hamimah Md Non, Salwa Hanim Zainal Alam for their valuable technical support, data collection and managing patients for MyEIRA study.

I wish to express my gratitude to my colleagues at the unit; Lena Nise, for your all your help especially in the beginning of my studies and also for helping me with SAS programming; Lina Palmlöf, for all the help and support given especially in dealing with Swedish agencies; Anne-Marie Wesley, for your sincere friendship and support throughout my time in Sweden; Max Vikström for statistical discussion and SAS programming advice as well as for other nice things; Xia Jiang for generous support, continuous understanding and who has always helped me; and to my roommates, Dong Yang, Mohammad Mohammadi, Hozan Hussein, Hedley Quintana and Ilais Moreno Velasquez, for your friendship and the enjoyable time we had together throughout these years. I would also like to express my thanks and appreciation to all my friends especially in the EIRA group and also from the corridor for their understanding and kind support and also thanks to everyone who were involved either directly or indirectly in my work.

To all the staff at the Embassy of Malaysia, Stockholm, Sweden and to all Malaysians in Stockholm, thank you so much for the warmest hospitality and making me feel like in Malaysia. To my dearest friends in Stockholm; Ejant and Rom, for the endless help and great time and memories, K Farizah and A Shahril, for their great hospitality especially when I first arrived in Sweden, K Yan and A Shahrin, for the great memories we had together, Fara and Mezan, for being so helpful and sharing great moments together, Shima and Kamarul for all nice time we shared. To my good friends in Uppsala; K Ami, thank you so much for always being there for me, all the help and especially the nice time we had during travelling, Mona, Duen and Makcik, for being so supportive and our nice time together.

To my very dear friends; Ros Shimah Nazri and Norfadzilah Juhari, a special thanks to you for being there for me especially during my hard time and giving me endless support and encouragement.

My deepest debt of gratitude is to my family; Kakcik, Bangcik, Hasif, Kak, A Majdi, Manja, Rahmat, Nurin, Sayang, Eibah, Wajat, K Lin, Abang, Kak Shidah, Intan, Durrah, Najieb, Nabiel and Zaki, for unlimited support, understanding and encouragement throughout the years of my study. To all my aunts, uncles and cousins; a million thanks for your support and encouragement.

The most special thanks I accord to my mum, Mak, for her unwavering understanding, selfless love, her encouragement and her “doa” throughout my life and especially during the preparation of this thesis.

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