Genetic variations in the NALP3 inflammasome
a susceptibility factor for inflammatory diseases

Deepti Verma
Abstract

Innate immunity has received impressive attention in the past decade owing to the discovery of the Toll like receptors (TLRs) and the NOD-like receptors (NLRs). While the TLRs specialize in fighting microbes at the cell surface, the NLRs complement by detecting and responding to intracellular microbes. Recently, the non-microbe sensing NLR called inflammasomes, have been identified, which senses metabolic stress as well as certain pathogenic microbes and elicits host’s inflammatory response.

The NLR, NALP3 (formerly known as cryopyrin) forms a large cytoplasmic complex called the ‘inflammasome’ when NALP3, activated by a stimuli, associates with the adaptor proteins ASC and CARD-8. This interaction leads to the activation of pro-inflammatory caspase-1 which subsequently results in the formation of Interleukin (IL)-1β and IL-18. Mutations in the gene encoding NALP3, termed NLRP3 can lead to its constitutive activation resulting in an uncontrolled production of IL-1β. These mutations have been implicated in hereditary inflammatory diseases, often grouped under cryopyrin associated periodic syndromes (CAPS).

This thesis describes a patient with a long history of arthritis and antibiotic resistant fever, but without the typical symptoms of CAPS. The patient was found to be a heterozygous carrier of two common polymorphisms Q705K in NLRP3 and C10X in the CARD-8. Experimental studies showed elevated levels of caspase-1 and IL-1β in the patient, and a total clinical remission was achieved by IL-1β blockade. These two polymorphisms combined, were found to occur in approximately 4% of the control population, suggesting the possibility of a genetic predisposition for inflammation in these individuals. Therefore, a cohort of rheumatoid arthritis (RA) patients, where elevated IL-1β could be one of the reasons behind chronic inflammation, was investigated. We found that carrying the combined polymorphisms resulted in increased RA susceptibility and a more severe disease course. Hypothetically, this subgroup of patients might benefit from IL-1β blockade. Additional studies are warranted to elucidate the functional effects of the two polymorphisms and to determine whether they identify a subgroup of patients that could benefit from IL-1 targeted therapy. Given the structural similarity of NALP3 to other NALPs, the possibility of involvement of the alternative, homologous genes cannot be eliminated.
List of original publications

This thesis is based on the following two papers, which will be referred to by their Roman numerals I and II:

I  
Gene Polymorphisms in the NALP3 Inflammasome are Associated with Interleukin-1 Production and Severe Inflammation. Relation to Common Inflammatory Diseases?  
*Arthritis Rheum* 2008; 58: 888-894

II  
**Kastbom A*, Verma D*, Eriksson P, Skogh T, Wingren G and Söderkvist P.**  
Genetic variation in proteins of the cryopyrin inflammasome influences susceptibility and severity of rheumatoid arthritis (the Swedish TIRA project).  
*Rheumatology* 2008; 47:415-417  
*These authors contributed equally to this work.*
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
</tr>
<tr>
<td>ASC</td>
<td>Apoptosis-associated speck like protein containing a CARD</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>CAPS</td>
<td>Cryopyrin-associated periodic syndrome</td>
</tr>
<tr>
<td>CARD</td>
<td>Caspase recruitment domain</td>
</tr>
<tr>
<td>CD</td>
<td>Crohn’s disease</td>
</tr>
<tr>
<td>CIAS1</td>
<td>Cold-induced autoinflammatory syndrome-1</td>
</tr>
<tr>
<td>CINCA</td>
<td>Chronic infantile neurological cutaneous and articular syndrome</td>
</tr>
<tr>
<td>CPPD</td>
<td>Calcium pyrophosphate dihydrate</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>DAMP</td>
<td>Danger-associated molecular patterns</td>
</tr>
<tr>
<td>DMARD</td>
<td>Disease-modifying anti-rheumatic drugs</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>FCAS</td>
<td>Familial cold-associated syndrome</td>
</tr>
<tr>
<td>FMF</td>
<td>Familial Mediterranean fever</td>
</tr>
<tr>
<td>ICE</td>
<td>Interleukin converting enzyme</td>
</tr>
<tr>
<td>IL-1Ra</td>
<td>Interleukin-1 receptor antagonist</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1β</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>LRR</td>
<td>Leucin rich repeats</td>
</tr>
<tr>
<td>MDP</td>
<td>Muramyl dipeptide</td>
</tr>
<tr>
<td>MSU</td>
<td>Monosodium urate</td>
</tr>
<tr>
<td>MWS</td>
<td>Muckle Wells syndrome</td>
</tr>
</tbody>
</table>
NACHT  Domain present in *Nais*, CIITA, HET-E (plant het product involved in vegetative incompatibility) and TP-1 (telomerase-associated protein)

NALP  NACHT-LRR-PYD containing protein

NLR  Nucleotide-binding domain and leucine-rich repeat containing gene family (NOD-like receptor according to old nomenclature)

NLRP3  NLR family, pyrin-containing domain 3

NOD  Nucleotide-binding and oligomerization domain

NOMID  Neonatal onset multisystem inflammatory disease

PAMP  Pathogen-associated molecular patterns

PRR  Pattern recognition receptor

PYD  Pyrin domain

PYPAAF  Pyrin-containing Apaf-1 like protein

RA  Rheumatoid arthritis

SE  Shared epitope

SNP  Single nucleotide polymorphism

TLR  Toll like receptors

TNF-α  Tumour necrosis factor-α

TRAPS  TNF-receptor associated periodic syndrome
## Contents

Introduction .................................................................................................................. 1  
Immune mechanisms: an overview ............................................................................. 1  
The NLR family: classification and nomenclature ......................................................... 3  
The NALP subfamily .................................................................................................... 4  
*NLRP3* gene and its mutations ...................................................................................... 5  
Inflammasomes ............................................................................................................ 6  
  Inflammasomes as sensors of pathogens and danger .................................................. 8  
  Inflammasome signaling and disease associations ...................................................... 9  
Rheumatoid arthritis and Inflammasome ...................................................................... 11  

Aims ............................................................................................................................. 13  
Material and Methods .................................................................................................. 14  
Results and Discussion ................................................................................................. 16  
Concluding remarks ...................................................................................................... 21  
Future directions .......................................................................................................... 22  
Acknowledgements ...................................................................................................... 24  
References ..................................................................................................................... 25
Introduction

Immune mechanisms: an overview

Immune responses are traditionally classified as either innate or adaptive. This classification is primarily based upon the distinct sensing and effector mechanism of each type of response. Briefly, the innate immune system results in the sensing of the pathogen directly at the site of infection and without the need of any previous exposure of host cells to these pathogens. The adaptive or the ‘acquired’ immune system allows for elimination of pathogens in late infection stage and results in building up of an immunological memory of the pathogen/antigen that remains encoded in the immune cells.

For several decades, the immunological studies have been focused mainly on adaptive immunity as the key player in immune regulation. The underlying reason being that adaptive responses were considered to provide protection against a vast repertoire of pathogens in a highly specific manner, while innate responses were regarded as primitive, of importance only in invertebrates, and moreover unspecific. However, there was a shift in the paradigm of immune regulation in the late nineties, when Toll protein, which previously was known for embryonal developmental function in *Drosophila melanogaster*, was shown to be essential for effective immune response against the fungi *Aspergillus fumigates* (Lemaitre, Nicolas et al. 1996). Two years later, in 1998, Toll Like Receptor 4 (TLR4) was cloned and shown to be the receptor for lipopolysaccharide (LPS), essential to mount an immune response against Gram-negative bacteria in a mouse model (Poltorak, He et al. 1998). Since then, there has been a great expansion of knowledge in the field of innate immunity and its role as a first line of defense against pathogens, thereby preceding and potentially modulating adaptive responses, has been appreciated.

Innate immunity relies on the host cell recognition of pathogens of certain conserved microbial motifs called pathogen associated molecular patterns (PAMPs), e.g. peptidoglycan in Gram-positive bacteria and lipopolysaccharide (LPS) in Gram-negative bacteria. PAMPs are recognized by the hosts ‘Pattern recognition receptors’ (PRRs), which are characterized by the presence of a ligand sensing, leucine rich receptor domain (LRR) and an effector domain (Uematsu and Akira 2006).
TLRs are among the first and one of the most well studied PRRs and to date, 10 TLRs (TLR1-TLR10) have been identified in humans (Beutler 2004). The TLRs can have a cell surface presence (TLR 1, 2, 4, 5, 6) or an endosomal presence (TLR 3, 7, 8 and 9) (Akira, Uematsu et al. 2006). Each TLR is known to recognize a set of microbial motifs in a wide range of organisms including bacteria, fungi, helminthes, protozoa and viruses (Beutler 2004). The discovery of TLRs was a key step forward in understanding host’s sensing of microbial pathogens and resulted in an accelerated understanding of innate responses. However, due to their superficial localization in the cell, they could not account for detection of the intracellular pathogens or the internal danger signals of host cells. Moreover, the TLR knockout mice models confirmed that TLRs alone could not be responsible for the cytokine response of the innate system (Beutler 2004).

The issue of detection of intracellular pathogens was resolved a few years later with the discovery of cytoplasmically located Nod-like receptors (NLRs), and the first NLRs to be cloned and intensely studied were Nod1 and Nod2 (Inohara, Koseki et al. 1999; Ogura, Inohara et al. 2001). The discovery of Nod1 was made when the intracellular, invasive pathogen *Shigella flexneri*, was shown to increase the activity of Nuclear factor-κB (NF-κB) in epithelial cells (Philpott, Yamaoka et al. 2000; Girardin, Tournebize et al. 2001). In the subsequent years, both Nod1 and Nod2 were shown to detect bacterial peptidoglycan (PGN) structures: meso-DAP, found in Gram-negative bacteria could be detected by Nod1 while muramyl dipeptide (MDP) found in both Gram-positive and Gram-negative bacteria could be detected by Nod2; both PGN structures being degradation products released from intracellular or phagocytosed bacteria (Chamaillard, Hashimoto et al. 2003; Girardin, Boneca et al. 2003; Girardin, Boneca et al. 2003; Inohara, Ogura et al. 2003). Nod1 and Nod2 act through activation of NF-κB, an important regulator of inflammatory response and hence are regarded as key mediators of immune response against bacteria and inflammation (Fritz, Ferrero et al. 2006).

Meanwhile, the knowledge that the endogenous danger signals could activate the innate immune system existed, though the mechanisms were not understood. Back in 1994, Polly Matzinger, proposed a so-called ‘danger model’ (Matzinger 1994), challenging Janeway’s classical hypothesis (Janeway 1992), that host cells tolerate self and reject whatever is foreign or non-self. Matzinger’s alternate hypothesis was that host cells, rather than attacking the non-self (in which case an embryo would also be rejected), reacts to the danger signals
released from dying cells and tissues (Matzinger 2002). These danger signals could arise from any trauma to host cells, for instance: exposure to UV or to extreme hot or cold temperatures, injury, infection and even certain drugs (Bianchi 2007). The endogenous signals were said to constitute the danger associated molecular patterns or ‘DAMPs’, more recently termed as ‘alarmins’, which have the property of directly activating the innate system and acting as a modulator of the adaptive system (Bianchi 2007).

The void in understanding of the innate systems potential sensors of ‘DAMPs’ or ‘alarmins’ was filled by the recent discovery of ‘inflammasome’-forming NLRs (Martinon, Burns et al. 2002). This subtype of NLRs are capable of forming a molecular scaffold which in addition to detecting microbial pathogens also sense a vast repertoire of endogenous stimuli, through a common pathway leading to the formation of inflammatory caspase and the cytokine, Interleukin-1β (IL-1β) (Martinon, Burns et al. 2002).

The NLR family: classification and nomenclature
Initially, the family members of the NLR family were referred to by a variety of names, like CATERPILLER, NACHT-LRR, NOD-LRR, CARD, NALP and PYPAF (Ting, Lovering et al. 2008). Recently, in order to bring consistency in referring to members of the NLR family, a new nomenclature was agreed upon (Ting, Lovering et al. 2008). NLR earlier stood for NOD-like receptors but according to the recent nomenclature, it stands for ‘nucleotide-binding-domain-and-leucine-rich-repeat-containing-gene-family-of-receptors’, which in humans consists of 22 members. These members show structural similarity in form of a C-terminal variable number of LRRs, a central nucleotide-binding domain (NBD:NACHT) and a N-terminal protein-protein-interaction domain consisting of either Caspase-activation-and-recruitment-domain (CARD), pyrin (PYD) or baculovirus-inhibitor-of-apoptosis-repeat (BIR) domain. The NLR family is broadly divided into 5 sub-members in NOD family, 14 members in NALP family, IPAF, NAIPs and CIITA. According to the most recent nomenclature, the pyrin containing subfamily has been named PAN, NALP and PYPAF, the genes belonging to this subfamily are referred to as NLRP. The members of the CARD containing subfamily has been named CARDs or NODs, the genes being referred to as NLRC, and the BIR containing subfamily has been named NAIP or BIRC, genes being referred as NLRB (Ting, Lovering et al. 2008).
The NALP subfamily

NALPs form the largest family of pyrin-containing proteins comprising of 14 members (NALP1-NALP14) (Tschopp, Martinon et al. 2003). The different NALPs vary in their expression patterns, for instance NALP1 is primarily expressed in heart, spleen, thymus, kidney and liver. NALP3 expression is confined to the immune cells and the NALP4, NALP5, NALP8 and NALP9 are expressed in gametes and preimplantation embryos (Tschopp, Martinon et al. 2003).

NALP1 and NALP2 were the first members to be identified in 2001, and were named so due to the presence of NACHT, LRR and PYD domains. The PYD domain in NALP1 can associate with the PYD domain of a 22 kDa pro-apoptotic adaptor protein called Apoptosis associated-Speck-like-protein-containing-a-CARD (ASC). PYD domain is implicated in pathways leading to apoptosis and inflammation (Martinon, Hofmann et al. 2001). NALP1 additionally possesses a unique C-terminal tail, comprising a CARD domain and a domain with unknown function, designated function-to-find-domain (FIIND) (figure A) (Martinon, Hofmann et al. 2001).

Interestingly, these two domains are also found in another protein, which was also discovered in 2001 and denoted TUCAN (Tumour-Upregulated-CARD-containing-Antagonist-of-caspase-Nine, and also known as CARD-8 or Cardinal) (Pathan, Marusawa et al. 2001). CARD-8 (the name used in this thesis) is found to be overexpressed in colorectal cancer tissue and shown to suppress cytochrome c/procaspase-9 mediated apoptosis (Pathan, Marusawa et al. 2001). In another study that came close to the heels of the first one, it was reported that CARD-8 could bind caspase-1 thereby inhibiting the formation of active IL-1β. Further, this group demonstrated that CARD-8 has an inhibitory effect on NF-κB regulation and when overexpressed it could in fact have a pro-apoptotic effect (Razmara, Srinivasula et al. 2002).

Returning back to the NALPs, another member to the NALP family was added with the identification of NALP3 (figure A), then termed as ‘Pyrin-containing-APAF-1-like-protein-1’ (PYPAAF1) (Manji, Wang et al. 2002). The authors showed that NALP3 associates with ASC using PYD domain interactions and could act as an upstream regulator of NF-κB. Incidentally, these studies coincided with the discovery of gene encoding NALP3 by another group, which alternatively denoted NALP3 as cryopyrin and called the gene CIASI (Hoffman, Mueller et al. 2001).
**Figure A.** Schematic illustration of NALP1 and NALP3 depicting their various domains. PyD, NACHT and LRR are common in both but NALP1 is characterized by its unique C-terminal FIIND-CARD domain.

For the sake of simplicity, the recent nomenclature (described in previous section) suggesting the term NALP3 (instead of cryopyrin, PYPAF1) for the protein, and *NLRP3* (instead of *CIAS1*) for the gene encoding NALP3, will be used.

**NLRP3 gene and its mutations**

*NLRP3*, the gene encoding NALP3 was discovered in 2001 by Hoffman and colleagues, while fine mapping the gene responsible for familial cold associated syndrome (FCAS) and Muckle Wells syndrome (MWS): two rare types of hereditary periodic fever syndromes (Hoffman, Mueller et al. 2001). The *NLRP3* gene was mapped to chromosome 1q44, it comprises nine exons, where exons 4–9 correspond to the LRR domain, exon three corresponds to NACHT domain and exon one corresponds to PYD domain (Hoffman, Mueller et al. 2001). Mutations in this gene were found in patients suffering from the above syndromes and all mutations found at that time were localized to exon three of the gene (Hoffman, Mueller et al. 2001; Hoffman, Gregory et al. 2003).

So far, only 3 more ‘non-exon-three mutations’ have been identified, which are present in LRR region (ISSAID 2003). Further, alterations in intron four and intron eight, probably leading to hypertension and MWS, respectively, have also been reported. In total, more than one hundred genetic alterations in *NLRP3* gene have been reported so far (ISSAID 2003). These mutations are systematically categorized as giving rise to syndromes broadly grouped under cryopyrin associated periodic syndromes (CAPS), also known as periodic fever syndromes. A detailed listing of the genetic alterations in the *NLRP3* gene and its associated symptoms can be found at ISSAID website.
NLRP3 gene was found to be intriguingly similar to MEVF gene, which is known for its role in familial Mediterranean fever. Both possess a PYD domain and are predominantly expressed in blood leukocytes. As PYD domain is found in a number of proteins that regulate apoptosis and inflammation, it is hypothesized that NLRP3, like MEVF could be involved in inflammation and apoptosis (Bertin and DiStefano 2000; Kastner and O'Shea 2001; Martinon, Hofmann et al. 2001; Manji, Wang et al. 2002).

Inflammasomes

The term inflammasome was coined by Tschopp and colleagues in 2002 to describe a multiprotein platform leading to the activation of caspase-1 and the subsequent formation of the pro-inflammatory cytokine, IL-1β (Martinon, Burns et al. 2002). Following the discovery of NALP1 in 2001, its ultimate function was demonstrated by its ability to form a multiprotein complex by recruiting additional proteins: caspase-1 at the CARD domain of ASC and caspase-5 at its C-terminal CARD domain. This complex, denoted as the ‘inflammasome’, leads to the activation of caspase-1, which cleaves pro-IL-1β and releases active IL-1β (Martinon, Burns et al. 2002). The selection of the term inflammasome was motivated by its similarity to another caspase-activating complex termed apoptosome, which triggers apoptosis (Martinon, Burns et al. 2002).

In 2004, it was shown by Agoistini et al. that NALP3 just like NALP1, could form an inflammasome by associating with ASC (figure B), leading to activation of caspase-1 and IL-1β (Agostini, Martinon et al. 2004). It was intriguing, since NALP3 lacked the C-terminal FIIND-CARD domain, which is present in NALP1. This was however explained by the association of NALP3 to CARD-8 instead, which possesses a FIIND-CARD domain and hence could compensate for the lack of this domain (Agostini, Martinon et al. 2004). Another important difference between the NALP1 and NALP3 inflammasomes is that, unlike NALP1, which recruits caspase-5 through CARD-CARD interactions at the C terminal, NALP3 only recruits caspase-1 twice, through CARD interactions involving ASC as well as CARD-8 (figure B).
Figure B. Schematic illustration of the NALP3 inflammasome complex consisting of NALP3, ASC and CARD-8 which brings two caspase-1 molecules together thereby causing its activation and leading to the formation of mature IL-1β from its inactive pro-form.

Caspase-1, like caspase-5 is pro-inflammatory in nature. Its substrates are pro-IL-1β, pro-IL-18 and possibly pro-IL-33, and its role is to cleave these inactive pro-forms to produce their mature active forms, which is the reason it is referred to as ‘interleukin-converting-enzyme’, ICE (Thornberry and Molineaux 1995; Schmitz, Owyang et al. 2005; Boraschi and Dinarello 2006; Martinon, Mayor et al. 2009). Much attention has been focused upon inflammasome-related formation of IL-1β and IL-18 (Stack, Beaumont et al. 2005; Piccini, Carta et al. 2008) but the role of IL-33 has not been well explored in this regard.

IL-1β is an extremely potent, pyrogenic cytokine essential to thwart pathogen attacks, so its unsolicited production by the inflammasome could be harmful, even fatal for the host (Beutler and Cerami 1987). To account for this, it is hypothesized that like the other NLR members, in a resting state, the LRR domain folds over the NACHT and pyrin domains, thereby auto-inhibiting inflammasome activity (Girardin and Yaniv 2001; Tschopp, Martinon et al. 2003). However, in the presence of a stimulus, LRR folds back, NALP3 oligomerizes and the adaptor molecule ASC is recruited through PYD-PYD interactions and CARD-8 is recruited through NACHT-FIIND interactions, to form the functionally active inflammasome complex (Kastner 2005; Mariathasan and Monack 2007). Another mechanism to tightly regulate the production of IL-1β is the requirement of two different signals (Dinarello 1996): The first
signal is through stimulus by TLR ligands like LPS, leading to the accumulation of 31 kDa inactive pro-IL-1β, while the second signal leads to activation of caspase-1 and cleavage of pro-IL-1β to the active 17 kDa IL-1β.

Interestingly, mutations in NLRP3 were among the first identified factors leading to the dysfunction of the inflammasome (Tschopp, Martinon et al. 2003). Most likely the need for a second signal is bypassed in the CAPS patients, who display a spontaneous production of IL-1β (Gattorno, Tassi et al. 2007).

**Inflammasomes as sensors of pathogens and danger**

Studies implicating a wide range of factors, including microbial PAMPs, exogenous non-microbial ligands as well as endogenous DAMPs as possible inflammasome stimuli have been reported, suggesting that inflammasome could be the long-sought danger sensing receptor.

The NALP3 inflammasome can respond to both bacterial and viral PAMPs (Kanneganti, Ozoren et al. 2006; Mariathasan, Weiss et al. 2006). Muramyl dipeptide (MDP), which activates Nod2 and increases NF-κB, is also known to activate caspase-1 and IL-1β through NALP3, suggesting that NALP3 could be an additional MDP sensor (Martinon, Agostini et al. 2004). Studies using a mouse model have shown that both NALP3 and Nod2 are essential for activation by MDP, indicating the possibility of a cross-talk between these two pathways (Pan, Mathison et al. 2007).

Gram-positive bacteria like *Staphylococcus aureus* as well as the presence of extracellular adenosine triphosphate (ATP) are a few of the triggers for inflammasome-dependent formation of mature IL-1β (Mariathasan, Weiss et al. 2006). Certain bacterial toxins, like α-toxin in *S. aureus* can result in the formation of pores thereby causing massive potassium efflux from the cell (Petrilli, Papin et al. 2007). ATP, released as a consequence of cell stress or damage, can form potassium channels by binding to P2X7 receptors leading to caspase dependent cleavage of pro-IL-1β to form mature IL-1β; this however requires a prior stimulation with a TLR ligand like LPS (Mariathasan, Weiss et al. 2006). Hence the common mechanism for bacterial toxins and ATP seems to be the ionic imbalance; in particular potassium efflux which acts as a potent danger signal recognized by the inflammasome (Perregaux and Gabel 1994; Mariathasan, Weiss et al. 2006; Petrilli, Papin et al. 2007).
Monosodium urate crystals (MSU) are formed when uric acid from dying cells combines with extracellular sodium, a condition typically observed in gout. These crystals constitute a ‘danger signal’ leading to NALP3 inflammasome mediated IL-1β release (Martinon, Petrilli et al. 2006). A similar inflammatory mechanism is observed in calcium pyrophosphate dehydrate (CPPD) crystals involved in pseudogout (Martinon, Petrilli et al. 2006). Several other factors such as alum adjuvants used in vaccines (Eisenbarth, Colegio et al. 2008), silica and asbestos particles in lung diseases (Cassel, Eisenbarth et al. 2008; Dostert, Petrilli et al. 2008), amyloid-β protein depositions in pathological conditions such as Alzheimer’s disease (Halle, Hornung et al. 2008), and most recently, hyaluronan, released from extracellular matrix during injury (Yamasaki, Muto et al. 2009), are among some of the identified activators of the NALP3 inflammasome.

**Inflammasome signaling and disease associations**

**Cryopyrin-associated periodic syndromes (CAPS)**

This category of diseases includes a trio of auto-inflammatory disorders: familial cold autoinflammatory syndrome (FCAS), Muckle Wells syndrome (MWS) and neonatal onset multiple inflammatory syndrome (NOMID); earlier known as chronic infantile neurological cutaneous articular syndrome (CINCA) (Hoffman, Mueller et al. 2001; Aganna, Martinon et al. 2002; Feldmann, Prieur et al. 2002; Hoffman, Gregory et al. 2003).

FCAS was first described in 1940 by Kile and Husk (Kile and Rusk 1940), and is the mildest form of CAPS characterized by recurrent fever, rash, arthralgia and conjunctivitis after exposure to cold. MWS was described in 1962 by Muckle and Wells (Muckle and Wells 1962) and patients with this syndrome show episodes similar to FCAS but the symptoms are not cold induced. Additionally, these patients may also display sensorineural hearing loss and systemic amyloidosis leading to renal failure (Muckle and Wells 1962). NOMID was described by Prieur et al. in the early 1980’s (Prieur and Griscelli 1981), and is the most severe form of CAPS involving chronic inflammation in the skin, joints and central nervous system, non-inflammatory manifestations in the form of cartilage overgrowth and hearing loss might also be present.
These three syndromes were initially defined as distinct clinical entities but later on revealed to have a close association, in the form of extensively overlapping symptoms. Recurrent fever, joint pain, urticaria-like rash and systemic inflammation are some of the common features which vary in degree of severity, FCAS being the mildest and NOMID being the most severe form. The overlapping phenotypes seen in patients have resulted in their being referred to as a clinical continuum, rather than distinct syndromes (Kastner 2005; Aksentijevich, C et al. 2007).

The genetic association became clear, when mutations in the NLRP3 gene were discovered in the patients suffering from these syndromes (Aganna, Martinon et al. 2002; Dode, Le Du et al. 2002; Feldmann, Prieur et al. 2002). So far, practically all these mutations have been reported in exon three, corresponding to the NACHT domain, suggesting the presence of functionally important sites in this region. No correlation between the position of mutation and disease severity have been found (Aksentijevich, Nowak et al. 2002), for instance M701T is identified as a MWS causing mutation while Q705K is postulated as unlikely to be pathogenic. Further, the same genetic alteration can lead to varying phenotypes, which could be attributed to environmental factors and genetic predisposition (Aganna, Martinon et al. 2002; Aksentijevich, Nowak et al. 2002). The disease causing mutations are believed to confer a gain-of-function to NLRP3 leading to an overly active inflammasome (Dowds, Masumoto et al. 2004). Consistent with this model, elevated IL-1β levels are detected in monocytes from patients with NLRP3 mutations (Aksentijevich, Nowak et al. 2002; Goldbach-Mansky, Dailey et al. 2006). The laboratory parameters of such patients indicate abnormally high values of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), both indicators of inflammation. The pivotal role of IL-1β is therefore suspected and also supported by the remarkable clinical improvement in these patients upon IL-1β blockade, using anakinra (Goldbach-Mansky, Dailey et al. 2006), which is a recombinant homolog of IL-1β receptor antagonist and acts as a competitive inhibitor of IL-1β binding to IL-1 receptor.

It is not clear how mutations impact the inflammasome activity, although 3-D structural modeling predicts that mutations can lead to surface disruption, which might affect the subdomain interactions within the NACHT domain or in certain cases provoke interactions with the LRR domain (Aksentijevich, C et al. 2007). Owing to these disruptions, the structural stability of the hypothetically closed state of NALP3 is disturbed and an opened state exposing the PYD domain and LRRs might be favored. The open state is probably the active
one which results in functional assembly of other components of inflammasome. (Aksentijevich, C et al. 2007).

**Rheumatoid arthritis and Inflammasome**

Rheumatoid arthritis (RA) is an inflammatory rheumatic disease characterized by chronic inflammation and subsequent destruction of synovial joints. The affected patients typically present with painful and swollen joints, most commonly in hands and feet, fatigue and occasional fever.

The diagnosis of RA patients is based on the 1987 American College of Rheumatology (ACR) classification criteria where at least 4 of the given below criteria needs to be fulfilled: morning stiffness for at least 1 hr, swelling (arthritis) of three or more joint areas, arthritis of proximal phalangeal or wrist joints, formation of rheumatic nodules, presence of rheumatic factor and radiographic erosions (Arnett, Edworthy et al. 1988).

The 28-joint Disease-Activity-Score (DAS28) is commonly used for assessing momentary disease activity in patients. It may also aid the rheumatologists to titer the dose of the given treatment if the patient is not optimally responding (Vander Cruyssen, Van Looy et al. 2005). DAS28 comprises swollen joint count (SJC), tender joint count (TJC), erythrocyte sedimentation rate (ESR) and the patient’s self assessment of general health (Prevoo, van ’t Hof et al. 1995). A DAS28 >5.1 is classified as high disease activity, 3.2 – 5.1 is moderate and < 3.2 is low disease activity (Fransen, Creemers et al. 2004).

Several pro-inflammatory cytokines like Tumor necrosis factor (TNF)-α, IL-1β, IL-15 and IL-17 have been implicated in the pathophysiology of RA (Bertolini, Nedwin et al. 1986; Rooney, Symons et al. 1990; Brennan, Maini et al. 1992; Arend and Dayer 1995). Among these, TNF-α and IL-1β are characterized as having most significant roles, and their elevated levels have been detected in RA patients (Rooney, Symons et al. 1990; Chu, Field et al. 1991). These cytokines are known to be regulated by the transcription factor NF-κB (Bondeson, Foxwell et al. 1999). In the recent years, an essential role of IL-18 as an inducer of TNF-α and IL-1β and as a mediator of angiogenesis in RA has also been suggested (Dai, Matsuno et al. 2004).
Today, RA is treated by the Disease-Modifying Anti-Rheumatic Drugs (DMARDs), like methotrexate and sulphasalazine, often in combination with low-dose corticosteroids (Svensson, Boonen et al. 2005; Kirwan, Bijlsma et al. 2007). The discovery that early aggressive DMARD therapy significantly improves disease outcome has brought about a great advancement in RA management (Nell, Machold et al. 2004). Further improvement in RA pharmacotherapy was achieved in the late nineties with the use of TNF antagonists, which effectively slow down the disease activity and disease progression, confirming this cytokine’s pivotal role in RA (Moreland, Baumgartner et al. 1997; Weinblatt, Kremer et al. 1999). Typically, if DMARDs fail to bring disease progression under control, TNF-inhibition becomes the alternative (Furst, Breedveld et al. 2005).

Owing to the elevated levels of IL-1β in RA patients, clinical trials using the IL-1β receptor antagonist (IL-Ra, anakinra) were performed in the late nineties. These studies suggested it to be beneficial against joint damage (Bresnihan, Alvaro-Gracia et al. 1998; Jiang, Genant et al. 2000). However, when compared to other biological treatments it resulted only in a modest improvement in the clinical parameters (Bresnihan, Alvaro-Gracia et al. 1998). Low efficacy of IL-1Ra was indicated in a Dutch RA cohort where only 14% of patients continued anakinra after 2 years (den Broeder, de Jong et al. 2006).

Recently discovered, NALP3 inflammasome, is involved in cleavage and secretion of IL-1β and IL-18, two important cytokines implicated in RA. However, only a couple of studies investigating the connection between NALP3 inflammasome and RA have so far been reported. The earliest study in this regard was done in 2005, where increased NALP3 expression in synovial tissue from RA patients was demonstrated (Rosengren, Hoffman et al. 2005). In another study involving four generations of a family diagnosed as suffering from CAPS, joint destruction, bone and cartilage lesions leading to disability, similar to RA was observed. Sequencing revealed a mutation (T348M) in NLRP3 gene, which has been implicated in MWS. Joint destruction, being an irreversible damage remained but other symptoms resolved upon treatment with anakinra (Lequerre, Vittecoq et al. 2007).
Aims
The general aim of the thesis was to elucidate the role of NALP3 inflammasome in chronic inflammatory conditions.

Specific aims were to:

1. Investigate the role of common genetic variants in the NALP3 inflammasome in a patient with chronic inflammatory condition. (Paper I).

2. Explore the role of genetic variations in the NALP3 inflammasome in RA susceptibility and severity (Paper II).
Material and Methods
The methods not described in Papers I and II are described here.

Genotyping
To specifically detect the Q705K and C10X alterations, MegaBACE SNuPe genotyping kit was used. This is a rapid and inexpensive method which is based upon single base primer extension. The specific primer is so designed that binds upstream to the single stranded DNA and ends precisely one base before the alteration site. A complementary fluorescence labeled dideoxy nucleotide is incorporated at the alteration site and detected on MegaBACE1000.

The PCR products were purified from excess primers and dioxxyriboonucleotide triphosphate (dNTPs) by treating with exonuclease I and shrimp alkali phosphatase (Exo-SAP-IT) for 15 minutes at 37°C followed by inactivation at 80°C. Purified product was mixed with SNuPe premix and a single nucleotide polymorphism (SNP) specific primer and thermally cycled at 96°C for 10 seconds, 57°C for 5 seconds, 60°C for 10 seconds, for a total of 26 cycles. The product was finally combined with formamide loading solution and a multi-injection marker for detection on MegaBACE1000 sequencing system.

Monocyte isolation
Peripheral blood was withdrawn from the study subjects and collected in heparin containing vacutainer tubes. Monocytes were isolated by using a density gradient centrifugation, i.e. Lymphoprep™ layered over Polymorphprep™. Blood was layered over the above gradient and centrifuged for 40 min at 480xg at room temperature. The band rich in mononuclear cells was collected and washed 3-4 times with KRG without Ca²⁺. After the final wash, the cells were resuspended in DMEM culture medium (supplemented with 25 mM Hepes, 100 U/mL of penicillin, and 100 µg/mL streptomycin). 2 x 10⁶ monocytes per well were seeded in a 12 well plate for 1.5 hrs. This was followed by washing to eliminate non adherent cells. For caspase-1 and IL-1β analysis, the cells were cultured overnight in DMEM supplemented with 10% human serum. For investigating NF-κB activation, monocytes were cultured for 9-11 days to differentiate into macrophages. DMEM culture medium was changed every 3 days.
Caspase-1 determination

Caspase-1 activity was determined using a commercial fluorometric kit. The monocytes were lysed, followed by the addition of a previously known caspase-1 substrate that is conjugated to a fluorescent reporter molecule. Upon encountering caspase-1 in the sample, the substrate molecule is cleaved and the fluorochrome is released. The fluorescence was detected on a chameleon multilabel detection platform. The level of caspase-1 activity in the cells is directly proportional to fluorescence intensity. Data was obtained as relative fluorescence units and represented as percentage of controls.

IL-1β determination

IL-1β levels were determined in plasma or monocyte supernatants using a commercially available LINCOplex kit. This kit relies upon a bead based flow cytometry method, where the reaction takes place on the surface of specific polystyrene microbeads. Each bead contains two red fluorochromes and the precise ratio of these fluorochromes allows the bead to be assayed. The specific capture antibody is attached to each bead set and is coupled to a PE-conjugated reporter antibody, which helps in detection of the sample. The analysis is performed on Luminex 100 (a dual laser flowcytometer), standard curves are generated for each analyte and values of samples are determined.

This method has a broad range of detection (0.06-2,000 pg/ml) and requires relatively low sample volumes. An additional feature of the method is that it can be used for simultaneous detection of other cytokines of interest within the same sample. However, due to the unavailability of IL-18 detection beads in combination with IL-1β, from the manufacturers, we restricted our analysis to just IL-1β determination.
Results and Discussion
This thesis describes two single nucleotide polymorphisms (SNPs), Q705K and C10X, in the genes NLRP3 and CARD-8, respectively. Proteins encoded by both these genes are parts of the NALP3 inflammasome that has the function of producing the pro-inflammatory cytokine IL-1β. The first SNP, Q705K, leads to an amino acid shift from glutamine (Q) to lysine (K) at the codon 705, corresponding to exon three, in the NLRP3. The second SNP, C10X leads to a change from cysteine (C) to a stop codon (X) at the codon 10, in CARD-8. We assessed the allele frequencies of these alterations in a DNA bank comprising of 806 control individuals. Allele frequency of Q705K in the above population was determined to be 6.5%, while that of C10X was 34%, due to which these are referred to as polymorphisms, rather than mutations, which are rare variations (< 1%). Approximately 4% of the control individuals simultaneously carried both the polymorphisms.

The Q705K found in NLRP3, has previously been reported, although alternatively numbered as Q703K (Hoffman, Gregory et al. 2003). Due to its prevalence in the control population, it has been suggested to be a non-pathogenic polymorphism (Hoffman, Gregory et al. 2003). Interestingly, two Muckle Wells syndrome-causing missense alterations, M701T and S710C, have been reported in its close proximity, however, in general, no relation between the alteration site and disease severity in CAPS patients has been observed. (Aksentijevich, C et al. 2007).

The C10X in CARD-8, results in a massive truncation of this normally 643 amino acid protein. Interestingly, this severe change is a common polymorphism that in the recent years has been associated with several inflammatory conditions, mainly related to disturbances in the NF-κB pathway. These associations are quite likely, since full length CARD-8 functions as an inhibitor of NF-κB, whereas this critical function seems to be abrogated in its truncated form (Fontalba, Martinez-Taboada et al. 2007), making it a highly probable candidate in inflammation. Crohn’s disease (CD), in a British cohort, was the first to be associated with C10X polymorphism (McGovern, Butler et al. 2006). This report prompted similar investigations on other CD cohorts and created a debate upon contradictory findings (Fisher, Mirza et al. 2007; Franke, Rosenstiel et al. 2007). More recently, an association with Alzheimer’s disease in women has been reported, suggesting possible sex-dependent mechanisms (Fontalba, Gutierrez et al. 2008).
The widespread distribution of these two polymorphisms in the control population makes it logical to assume that carrying one of these *per se* should not constitute any risk, which is also suggested in the case of Q705K (Hoffman, Gregory et al. 2003; Aksentijevich, C et al. 2007); However, it is tempting to speculate that both the polymorphisms in inflammasome components taken together, could significantly contribute to its dysregulation leading to elevated IL-1β levels. Alternatively, either of these could suffice as a disease causing alteration, triggered by a microbial infection or an endogenous danger signal.

The results from Paper I suggest a possible role of the above polymorphisms in the pathogenesis of a 29 year-old patient, suffering from arthritis and chronic febrile sickness. The investigations in Paper II were done with the intention of elucidating the role of these polymorphisms, in common, chronic inflammatory conditions, like RA. Results from these investigations indicated an increased RA susceptibility and a more severe disease course in the patients who possess both the polymorphisms.

**Paper I**

The patient in this study showed strikingly elevated laboratory parameters of inflammation: CRP, ESR and white blood count, and suffered from frequent bouts of high fever in conjunction with rashes. The characteristic symptoms of CAPS patients like short stature, urticaria, bony overgrowth of joints or neurological involvement were absent.

The patient had been healthy until eight years of age and the symptoms debuted after a severe *Streptococcal* infection. It may be speculated, that this infection, coupled with abnormal inflammasome component(s), might have triggered the inflammatory cascade, as observed in the CAPS patients who get inflammatory attack upon encountering the most trivial stimuli (Hoffman, Rosengren et al. 2004; Rosengren, Mueller et al. 2007). However, with a few exceptions including our patient, all CAPS patients report disease onset at birth or infancy (Shinkai, McCalmont et al. 2008).

No clinical benefit was achieved by resorting to TNF-α blockade, after corticoids and DMARDs had only been partially successful or failed. A central role of IL-1β in the pathogenesis of this patient was suspected. Since mutations in the *NLRP3* have previously been implicated in an increased IL-1β production, we decided to investigate the NALP3
inflammasome genes in this patient. The two above mentioned polymorphisms (Q705K and C10X) were detected.

Our experimental results are in line with our hypothesis, and elevated basal levels of both caspase-1 and IL-1β were detected in the patient compared to healthy volunteers. These data suggest a constitutive activity of the inflammasome, which is in agreement with previous studies reporting elevated IL-1β levels in the CAPS patients possessing gain-of-function mutations (Martinon, Mayor et al. 2009). Upon stimulation with S. aureus, the patient's monocytes showed a more pronounced increase in IL-1β compared to the controls. Healthy individuals however, require an additional stimuli for a more remarkable IL-1β production (Netea, Nold-Petry et al. 2009), as was observed by our controls' modest IL-1β secretion when only stimulated with S. aureus. Further, a combination of prior TLR stimulation using LPS, followed by S. aureus resulted in strikingly increased IL-1β levels in both controls as well as the patient. According to previous data, in CAPS patients, the need of second stimuli is bypassed and no remarkable increase upon a second stimuli is observed, which is attributed to 'secretory exhaustion' of the inflammasome (Gattorno, Tassi et al. 2007). Contrary to these, we observed a dramatic increase with S. aureus treatment, after overnight stimulation with LPS. This could be due to differences in methods of LPS stimulation as well as the choice of the second stimuli (S. aureus vs. ATP). Our healthy volunteers displayed similar or even higher IL-1β levels compared to the patient, after the two stimuli, which can be attributed to individual differences between the control subjects (Gattorno, Tassi et al. 2007).

Our results also highlight the effect of anakinra on spontaneous as well as induced IL-1β secretion in the patient's monocytes, which is in agreement with the complete clinical remission in the patient following treatment. However, the decreased IL-1β response, observed upon bacterial challenge could have possible implications on resistance to certain infections, in the patients receiving anakinra treatment.

Interestingly, in several CAPS patients, only potentially low penetrance alterations, so-called due to their presence in non-symptomatic relatives, have been detected (Aksentijevich, C et al. 2007). Similarly, the Q705K has also been detected in symptomatic as well as non-symptomatic individuals, which is why it has previously been classified as a polymorphism with a low penetrance (Hoffman, Gregory et al. 2003; Aksentijevich, C et al. 2007). We as well, found this alteration to occur at an allele frequency of approximately 6% in the normal population. This raises the question whether it's possible that such asymptomatic individuals
are more susceptible for inflammatory conditions that might be triggered upon encountering the ‘right’ stimuli? It would also be of interest to elucidate if those CAPS patients, where no mutation is detected, additionally possess a C10X in \textit{CARD-8}.

Hereditary periodic fever syndromes include, in addition to CAPS, familial Mediterranean fever (FMF), TNF-receptor-associated-periodic-syndromes (TRAPS), Hyperimmunoglobulinemia D-with-periodic-fever-syndrome (HIDS). All these conditions are characterized by recurrent unprovoked fever and inflammation (Kastner 2005). The pathogenesis of each disease is different, FMF being associated with dysregulations in NF-κB and IL-1β, TRAPS with TNF-α and HIDS with deficiency of mevalonate kinase (Kastner 2005). We investigated the coding regions of these three genes as well, to rule out the possibility of their involvement. However, no pathogenic alterations in above genes were detected.

\textbf{Paper II}

Following the experimental studies in the first paper, which confirmed our suspicions regarding the involvement of IL-1β, coupled with the total clinical remission upon its blockade, we further hypothesized, that these two polymorphisms may imply a genetic susceptibility for chronic inflammatory disorders. One such disease, where the pathogenic role of IL-1β has long been established, is RA: therefore a well characterized cohort of RA patients with a follow-up period of at least 5 years, was investigated.

Early diagnosis and treatment are regarded as key elements in controlling RA disease progression (Quinn, Conaghan et al. 2001). Owing to the insufficiency of DMARDs to treat RA, a number of other biological treatments targeting the pro-inflammatory cytokines have been proposed and their effects studied in the clinical trials. The outcomes using IL-1β directed therapies have not been very promising and as a consequence, anakinra is not used as the first line of treatment against RA. However, the clinical trials with anakinra indicate that a subset of patients shows a clear improvement in symptoms (den Broeder, de Jong et al. 2006) and hence a timely intervention in this subset, if they could be identified, would be highly desirable.

The TIRA (Swedish acronym for ‘early interventions in rheumatoid arthritis’) cohort comprises RA patients enrolled according to the criteria described in Paper II. Investigations
on this cohort revealed that being a carrier of either of these polymorphisms individually did not confer any risk for RA. However their combination constituted a significantly increased risk for RA (O.R=2.2, P= 0.04). This association was particularly evident in the patients that were SE/ anti-CCP positive (O.R=3.5, P= 0.005) both of which are markers of an aggressive disease course in RA (Berglin, Padyukov et al. 2004).

An interesting observation was that the fraction of patients (13%) with a severe disease activity indicated by the prescription of TNF-blocking therapy, possessed an altered NLRP3 or CARD-8 genotype. Moreover, the probability of insufficiency of DMARDs, alternatively risk for receiving TNF-blockade, strikingly increased in individuals where both the genetic alterations were present. This raises the question whether IL-1β plays a central role in the pathogenesis of this subgroup of individuals, and whether these individuals would have benefitted from IL-1β blockade.

Since the number of individuals who were prescribed TNF-blockade (n=23) was quite small in our study, it would be of interest to elucidate these finding in additional cohorts.
Concluding remarks
This thesis presents an intriguing and novel hypothesis that polymorphisms in the inflammasome genes could contribute to increased susceptibility for certain common, chronic inflammatory conditions, as shown in case of the individual patient (Paper I) and in the RA cohort (Paper II). If confirmed, this finding would imply that individuals who simultaneously carry these two polymorphisms would be at increased risk for inflammation upon encountering the stimuli which provokes their inflammasome. At the same time, it also suggests that such subgroup of individuals would be more likely to benefit from IL-1β blockade. For instance in Paper I, the patient who suffered from ‘diffuse’ inflammatory symptoms, in the sense that his symptoms could not be clearly categorized under CAPS, spondylarthritis or Still’s disease, and where conventional treatments showed limited response, IL-1β blockade using anakinra was very successful. This also implies that such patients could benefit from early genetic determination followed by a timely intervention with IL-1β directed therapy. Accordingly, identifying the subgroup of RA patients through genetic screening, who would most likely benefit from IL-1β blockade, could prevent irreversible joint damage and suffering.
Future directions

Our data may not obviously be translated as a direct effect of the two polymorphisms; it should rather be appreciated as a plausible hypothesis of polymorphism(s) as a cause of aberrant IL-1β production leading to chronic inflammation. It is essential that the findings are confirmed by functional studies of the described polymorphisms using genetic constructs to determine their causal effects. It would also be of interest to investigate the role of these polymorphisms in other chronic inflammatory conditions which are known to involve IL-1β.

NALPs are a family of structurally similar proteins and NALP3 shows high structural resemblance to NALP1, NALP5, NALP10 and NALP12 (Tschopp, Martinon et al. 2003). NLRP1 gene polymorphisms have mainly been associated with autoimmune diseases like vitiligo and type-1 diabetes (Jin, Mailloux et al. 2007; Magitta, Boe Wolff et al. 2009), its role in CAPS patients has not been well elucidated. Recently, NLRP12 gene alterations have also been implicated in CAPS patients (Jeru, Duquesnoy et al. 2008). Additionally, polymorphisms in the promoter region of NLRP3 have been detected and suggested to be involved in pathogenesis of mutation negative CAPS patients (Anderson, Mueller et al. 2008). Other regulatory regions of NLRP3 have been shown to be associated with increased susceptibility to CD (Villani, Lemire et al. 2009). Probably, investigation of these alternative sites on NLRP3 and/or its homologus genes might provide a better insight into the pathogenesis of chronic inflammatory conditions.
Popular science description
Vertebrates possess a sophisticated immune system that helps them not only to fight against a wide variety of microbes but also against non-microbial harmful substances. This ability partly exists from birth, denoted ‘innate immunity’ and is further acquired upon being exposed to the pathogens, in which case it is retained for life, and classified as adaptive immunity. Innate immunity has been intensely studied in the past few decades, and has led to a better understanding of the mechanisms, behind how individuals recognize and respond when exposed to pathogens. Several types of host’s sensors have been identified, each of which is specialized for detecting a particular type of microbe.

Recently a special type of sensor termed as the ‘inflammasome’ was identified, which in addition to microbes, recognizes harmful particles, in the environment as well as inside the body. The inflammasome is made up of three different proteins which upon sensing danger, associate together to form a complex. This complex then responds by producing inflammatory substances, most important of which is Interleukin-1. However, in certain cases the inflammasome complex can erroneously result in inflammation in the host by continuously producing Interleukin-1. An example of the above situation is the genetic alterations in the inflammasome proteins which causes unsolicited Interleukin-1 production, and thereby lead to serious inflammatory outcomes. Patients suffering from such defects are successfully treated with the blockade of Interleukin-1.

We report a patient who had been suffering from severe inflammation for 20 years. Genetic screening of the inflammasome components revealed two variants which may have contributed to his symptoms. Upon treatment with IL-1 blockade the patient showed remarkable recovery from the symptoms and the results from our experimental studies indicated an over-activity of the inflammasome. The two genetic alterations present in this patient occur in approximately 4% of the normal population implying that these individuals may be more prone to develop inflammatory diseases. We therefore genetically investigated a group of patients with rheumatoid arthritis, which is a joint disease caused by long-term inflammation. We found that the patients, who carried both the genetic variants, were at increased risk for rheumatoid arthritis and required more aggressive forms of treatment. Our results suggest that such individuals could possibly benefit from an early genetic screening and a timely initiation of IL-1 blocking treatment. More studies in other RA groups need to be done to confirm these results.
Acknowledgements
I would like to express my sincere gratitude and appreciation to:

My supervisor, Peter Söderkvist, for giving me the opportunity to work on this project. It has been a great learning experience and this is just the beginning!

My co-supervisors, Per Eriksson, Eva Särndahl and Maria Lerm for the expert guidance and encouragement.

All members in our group, Annette Molbaek, Asa Schippert, Lena Thunell, Nils Elander and Jonas Ungerbäck for always willing to help and taking time to answer my queries. It’s great to work with you guys!

My collaborators, Alf Kastbom, Ida Schoultz and Jan-Ingvar Jönsson, for past collaborations as well as future projects.

My parents, for always encouraging me. My husband Sanjay and sons Manu and Aditya for all the positive energy and for making everything worthwhile!
References


