Kari Luotola

ASSOCIATIONS OF INTERLEUKIN-1 (IL-1) GENE VARIATION AND IL-1 RECEPTOR ANTAGONIST PHENOTYPES WITH IMMUNOLOGICAL RESPONSES, METABOLIC DYSREGULATION AND TYPE 2 DIABETES

ACADEMIC DISSERTATION

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... 
The wind it was so insistent
With tales of a stormy south
...
I walked out this morning
It was like a veil had been removed from before my eyes
For the first time I saw the work of heaven
...
And inside every turning leaf
Is the pattern of an older tree
The shape of our future
The shape of all our history
And out of the confusion
Where the river meets the sea
Came things I'd never seen
...
I know it's true
It's written in a sky as blue
As blue as your eyes, as blue as your eyes
If nature's red in tooth and claw
Like winter's freeze and summer's thaw
...
Where the river meets the sea
Something new would arrive
Something better would arrive
...

Sting
ABSTRACT

The upstream proinflammatory interleukin-1 (IL-1) cytokines, together with a naturally occurring IL-1 receptor antagonist (IL-1Ra), play a significant role in several diseases and physiologic conditions. The IL-1 proteins affect glucose homeostasis at multiple levels contributing to vascular injuries and metabolic dysregulations that precede diabetes. An association between IL-1 gene variations and IL-1Ra levels has been suggested, and genetic studies have reported associations with metabolic dysregulation and altered inflammatory responses.

The principal aims of this study were to: 1) examine the associations of IL-1 gene variation and IL-1Ra expression in the development and persistence of thyroid antibodies in subacute thyroiditis; 2) investigate the associations of common variants in the IL-1 gene family with plasma glucose and insulin concentrations, glucose homeostasis measures and prevalent diabetes in a representative population sample; 3) investigate genetic and non-genetic determinants of IL-1Ra phenotypes in a cross-sectional setting in three independent study populations; 4) investigate in a prospective setting (a) whether variants of the IL-1 gene family are predictors for clinically incident diabetes in two population-based observational cohort studies; and (b) whether the IL-1Ra levels predict the progression of metabolic syndrome to overt diabetes during the median follow-up of 10.8 and 7.1 years.

Results from on patients with subacute thyroiditis showed that the systemic IL-1Ra levels are elevated during a specific proinflammatory response and they correlated with C-reactive protein (CRP) levels. Genetic variation in the IL-1 family seemed to have an association with the appearance of thyroid peroxidase antibodies and persisting local autoimmune responses during the follow-up. Analysis of patients suffering from diabetes and metabolic traits suggested that genetic IL-1 variation and IL-1Ra play a role in glucose homeostasis and in the development of type 2 diabetes. The coding IL-1 beta SNP rs1143634 was associated with traits related to insulin resistance in cross-sectional analyses. Two haplotype variants of the IL-1 beta gene were associated with prevalent diabetes or incident diabetes in a prospective setting and both of these haplotypes were tagged by rs1143634. Three variants of the IL-1Ra gene and one of the IL-1 beta gene were consistently identified as significant, independent determinants of the IL-1Ra phenotype in...
two or three populations. The proportion of the phenotypic variation explained by the genetic factors was modest however, while obesity and other metabolic traits explained a larger part. Body mass index was the strongest predictor of systemic IL-1Ra concentration overall. Furthermore, the age-adjusted IL-1Ra concentrations were elevated in individuals with metabolic syndrome or diabetes when compared to those free of metabolic dysregulation. In prospective analyses the systemic IL-1Ra levels were found as independent predictors for the development of diabetes in people with metabolic syndrome even after adjustment for multiple other factors, including plasma glucose and CRP levels. The predictive power of IL-1Ra was better than that of CRP. The prospective results also provided some evidence for a role of common IL-1 alpha promoter SNP rs1800587 in the development of type 2 diabetes among men and suggested that the role may be gender specific. Likewise, common variations in the IL-1 beta coding region may have a gender specific association with diabetes development.

Further research on the potential benefits of IL-1Ra measurements in identifying individuals at high risk for diabetes, who then could be targeted for specific treatment interventions, is warranted. It has been reported in the recent literature that IL-1Ra secreted from adipose tissue has beneficial effects on glucose homeostasis. Furthermore, treatment with recombinant human IL-1Ra has been shown to have a substantial therapeutic potential. The genetic results from the prospective analyses performed in this study remain inconclusive, but together with the cross-sectional analyses they suggest gender-specific effects of the IL-1 variants on the risk of diabetes. Larger studies with more extensive genotyping and resequencing may help to pinpoint the exact variants responsible and to further elucidate the biological mechanisms for the observed associations. This would improve our understanding of the pathways linking inflammation and obesity with glucose and insulin metabolism.
TIIVISTELMÄ
Interleukiini-1 (IL-1) sytokiiniperhe on voimakas tulehdusvasteen induktori sekä säätelijä – sisältäen luonnollisen IL-1 reseptorin antagonistin (IL-1Ra) – ja on siten merkittävä tekijä useissa eräissä sairauksissa sekä fysiologisissa tilanteissa. IL-1 proteiinit vaikuttavat verensokeritasapainoon useilla eri tasoilla ja tulehdusreaktiota voimistavat IL-1 sytokiinit kiíhdyttävät verisuonten vauriomekanismeja sekä epäedullisia aineenvaihdunnan säätelymekanismeja edeltäen diabeteksen kehittymistä. Aiemmat tutkimukset ovat viitanneet siihen, että IL-1 perheen geneettinen vaihtelun asosioituu IL-1Ra tasoihin. Useat geneettiset tutkimukset ovat myös osoittaneet yhteyden epäedullisten metabolisten säätelymekanismien ja poikkeavan immuunivasteen välillä.

Tutkimuksen tavoitteet olivat: 1) tutkia IL-1 perheen geneettisen vaihtelun sekä IL-1Ra:n pitoisuksien assosiaatiota kilpirauhasvasta-aineiden kehittymiseen ja pysyvyyteen subakuuttissa kilpirauhasvastulehdussa; 2) tutkia IL-1 geeniperheen yleisten varianttien assosiaatiota glukoosi ja insuliinipitoisuksiin, glukoositasapainon osoittimiin sekä diabeteksen esiintyvyyteen edustavassa väestöotoksessa; 3) tutkia IL-1Ra fenotyypin geneettisiä ja ei-geneettisiä determinantteja poikkileikkausasetelmassa kolmessa riippumattomassa tutkimuspopulaatiossa; 4) tutkia etenevää asetelmassa (a) ennustavatko IL-1 geeniperheen variantit kliinisesti havaitun diabeteksen ilmaantuvuutta kahdessa väestöpohjaisessa kohortiainestossa; sekä (b) ennustavatko IL-1Ra:n pitoisuudet diabeteksen kehittymistä henkilöillä, joilla on todettu metabolinen oireyhtymä mediaanisellä seuranta-aikoina 10.8 ja 7.1 vuotta.

Plasman IL-1Ra:n pitoisuudet havaittiin kohonneiksi subakuuttia kilpirauhastulehdusta sairastavilla potilailla sekä korreloivan C-reaktiivisen proteiinin (CRP) pitoisuksiin kanssa. IL-1 geenien vaihtelu näytti asosioituvan mitattavien tyreoiideperoksidaasi vasta-ainetasojen kanssa sekä pysyvymänen kilpirauhasen autoimmiunivasteiden kanssa seuranta-aikana. Diabetesta ja metabolisista ominaisuuksia koskevat tututkoit viittasivat siihen, että IL-1 geenivaihtelulla sekä IL-1Ra:lla on merkittävä rooli glukoositasapainon säätelyssä sekä tyypin 2 diabeteksen kehittymisessä. IL-1 beta geenin koodaavan alueen yhden emäsparin polymorfin SNP rs1143634 havaittiin asosioituvan insuliiniresistenssii liittyviin ominaisuuksiin. Vastaavasti kaksi IL-1 beta geenin haplotyyppia asosioituivat diabeteksen ilmaantuvuuteen tai esiintyvyyteen; ja
molemmat haplotypit sisälsivät edellä mainitun rs1143634 harvinaisemman alleelin. Kolme IL-1 reseptori antagonisti geenin ja yksi IL-1 beta geenin varianttia assosioituivat tilastollisesti merkittävästi ja useista muista tekijöistä riippumatta IL-1Ra pitoisuuteen kahdessa tai kolmessa tutkimuspopulaatiossa. Geneettisten tekijöiden selitysosuus IL-1Ra fenotyyppin vaihtelussa oli kuitenkin melko vaatimatonta, sillä obesiteetti ja muut metabolism set ominaisuudet selittivät fenotyyppin variaatiota geneettisiä tekijöitä enemmän. Ikäväkioidut IL-1Ra tasot olivat korkeampia niillä henkilöillä, joilla todettiin metabolinen oireyhtymä tai diabetes verrattuna niihin joilla näitä aineenvaihdunta-häiriöitä ei havaittu. Etenevässä tutkimusasetelmassa IL-1Ra pitoisuuksien havaittiin itsenäisesti ennustavan diabeteksen kehittymistä niillä henkilöillä, joilla oli metabolinen oireyhtymä lähőtilanteessa, riippumatta useista muista tekijöistä kuten esimerkiksi veren glukoosista ja CRP pitoisuuksista. IL-1Ra tasojen ennusteellinen merkitys oli vahvempi kuin CRP:n. Etenevä tutkimus tuotti viitteellistä näyttöä IL-1 alfa geenin promoottorialueen yleisen variantin rs1800587 osuudesta tyypin 2 diabeteksen kehittymisessä miehillä ja havaittu vaikutus saattaa olla sukulaisesta riippuvaa. Vastaavasti IL-1 beta variantin rs1143634 vaikutus tyypin 2 diabeteksen riskiin saattaa olla sukulaisesta riippuvainen.

# 1. INTRODUCTION

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<tbody>
<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
</tr>
<tr>
<td>AGE</td>
<td>advanced glycoation endproduct</td>
</tr>
<tr>
<td>AIRGENE</td>
<td>Air Pollution and Inflammatory Response in Myocardial Infarction Survivors: Gene-Environment Interaction in a High Risk Group</td>
</tr>
<tr>
<td>AITD</td>
<td>autoimmune thyroid disease</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>Ca++</td>
<td>calcium ion</td>
</tr>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CD4+</td>
<td>cluster of differentiation 4 glycoprotein expressed on T helper cells</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>CEU</td>
<td>Utah residents with Northern and Western European ancestry from the Centre d’ Etude du Polymorphisme Humain collection</td>
</tr>
<tr>
<td>cGMP</td>
<td>cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>CHD</td>
<td>coronary heart disease</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cell</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetra-acetic acid</td>
</tr>
<tr>
<td>ERK</td>
<td>extracellular signal regulated kinase</td>
</tr>
<tr>
<td>ESR</td>
<td>erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>FEIA</td>
<td>fluoro enzyme immunoassay</td>
</tr>
<tr>
<td>FFA</td>
<td>free fatty acid</td>
</tr>
<tr>
<td>G-CSF</td>
<td>macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>granulocyte-macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>GWAS</td>
<td>genome-wide association study</td>
</tr>
<tr>
<td>HapMap</td>
<td>the international haplotype map project of human genome</td>
</tr>
<tr>
<td>HbA1c</td>
<td>glycated haemoglobin</td>
</tr>
<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
</tr>
<tr>
<td>HLA</td>
<td>human leucocyte antigen</td>
</tr>
<tr>
<td>HOMA</td>
<td>homeostasis model assessment (for insulin resistance - HOMA-IR)</td>
</tr>
<tr>
<td>HR</td>
<td>hazard ratio</td>
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</tbody>
</table>
ICE interleukin-converting enzyme
IDF International Diabetes Federation
IFG impaired fasting glucose
IFNα interferon alpha
IFNγ interferon gamma
Ig immunoglobulin
IgG immunoglobulin G
IGT impaired glucose tolerance
IKKβ Iκβ kinase beta
IL1A interleukin-1 alpha gene
IL-1α interleukin-1 alpha
IL1B interleukin-1 beta gene
IL-1β interleukin-1 beta
IL-1Ra interleukin-1 receptor antagonist
IL1RN interleukin-1 receptor antagonist gene
IL1RN*2 minor allele 2 of IL1RN gene
IL-4 interleukin 4
IL-6 interleukin 6
IRS-1 insulin receptor substrate -1
Iκβ I kappa beta
JNK c-Jun N-terminal kinase
K+ potassium ion
kb kilobase
kDa kilodalton
LD linkage disequilibrium
LDL low-density lipoprotein
LOD logarithm of the odds
LPS lipopolysaccharide
MAF minor allele frequency
MAPK mitogen-activated protein kinase
MCP-1 monocyte chemotactic protein-1
M-CSF macrophage colony-stimulating factor
MetS metabolic syndrome
MI myocardial infarction
MODY1 Maturity Onset Diabetes of the Young type 1
MODY3 Maturity Onset Diabetes of the Young type 3
mRNA messenger ribonucleic acid
MyD88 myeloid differentiation primary response gene 88
NF-κB nuclear factor κB (kappa-light-chain-enhancer of activated B cells)
NH2- amine
<table>
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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>NLR</td>
<td>NOD-like receptor</td>
</tr>
<tr>
<td>NOD</td>
<td>nucleotide binding oligomerization domain</td>
</tr>
<tr>
<td>OGTT</td>
<td>oral glucose tolerance test</td>
</tr>
<tr>
<td>P2X7</td>
<td>purinoreceptor 7</td>
</tr>
<tr>
<td>PAMP</td>
<td>pathogen-associated molecular patterns</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PKC</td>
<td>protein kinase C</td>
</tr>
<tr>
<td>PRR</td>
<td>pattern recognition receptors</td>
</tr>
<tr>
<td>RAGE</td>
<td>receptor for advanced glycosylated endproduct</td>
</tr>
<tr>
<td>RAS</td>
<td>rennin–angiotensin system</td>
</tr>
<tr>
<td>RIA</td>
<td>radioimmunoassay</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RR</td>
<td>risk ratio</td>
</tr>
<tr>
<td>SAT</td>
<td>subacute thyroiditis</td>
</tr>
<tr>
<td>SHBG</td>
<td>sex hormone-binding globulin</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>T2D</td>
<td>type 2 diabetes</td>
</tr>
<tr>
<td>TG</td>
<td>thyroglobulin</td>
</tr>
<tr>
<td>TGFβ</td>
<td>transforming growth factor beta</td>
</tr>
<tr>
<td>Th1</td>
<td>T-helper cell type 1</td>
</tr>
<tr>
<td>Th17</td>
<td>T-helper cell type 17</td>
</tr>
<tr>
<td>Th2</td>
<td>T-helper cell type 2</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNFα</td>
<td>tumour necrosis factor alpha</td>
</tr>
<tr>
<td>TPO</td>
<td>thyroid peroxidase</td>
</tr>
<tr>
<td>TSH</td>
<td>thyrotropin</td>
</tr>
<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
</tr>
<tr>
<td>VNTR</td>
<td>variable number tandem repeat</td>
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their Roman numerals:

I  Luotola K, Mantula P, Salmi J, Haapala AM, Laippala P, Hurme M. Allele 2 of interleukin-1 receptor antagonist gene increases the risk of thyroid peroxidase antibodies in subacute thyroiditis. APMIS. 2001;109:454-60. (Previously unpublished data is also included in addition to this publication)


1. INTRODUCTION

Interleukin-1 (IL-1) cytokines are upstream mediators of proinflammatory responses affecting antigen recognition patterns and lymphocyte function. IL-1 alpha (IL-1α) and beta (IL-1β) are capable of evoking a wide variety of biological effects as a host response to trauma or infection and play a significant role in several diseases and physiologic conditions including diabetes; cardiovascular diseases; thyroiditis; bone, joint and cartilage disorders; neuroendocrine metabolism and reproductive biology. The IL-1 family proteins affect glucose homeostasis in pancreatic beta cells, adipose tissue and at central levels. A prolonged exposure to IL-1β has been shown to lead to a reduction of insulin-induced glucose uptake in adipocytes and is involved in the initiation and progression of atherosclerosis. Treatment with recombinant human IL-1 receptor antagonist (IL-1Ra) improves glycaemic control in type 2 diabetes (T2D), while increased expression and secretion of the natural IL-1Ra from adipose tissue has previously been shown to associate with obesity, insulin resistance and preceding the development of T2D.

The IL-1 type I receptor has a high level of homology with the cytoplasmic domains of Toll-like receptors. IL-1β is capable of activating the cytoplasmic signalling complexes, called inflammasomes, together with other factors that augment the secretion of active IL-1β. The proinflammatory activity of IL-1 cytokines is tightly regulated by multiple mechanisms including the natural antagonist, IL-1Ra. An association between the genetic variation of IL-1 and IL-1Ra levels has been proposed in previous studies. The models of genetic deletion of IL-1Ra have been associated with low weight gain and metabolic dysregulation. In humans, recent reports have described a severe human inherited inflammatory syndrome caused by defects in the IL-1Ra gene.

Metabolic regulation and immune responses are central homeostatic mechanisms that are highly integrated and dependent on each other. The inflammatory factors are commonly known to have an influence on glucose homeostasis and they contribute to the vascular injury and metabolic dysregulation that precedes diabetes. In candidate gene studies the identification of potentially causal genetic loci or closely related variants requires biological plausibility in the development of the disease when showing substantial associations with complex traits such as T2D. Furthermore, allelic dose–response relationships across different populations are important considerations and
consistent replication in different populations markedly increases the evidence for causality. A previous linkage study has revealed a strong signal in the candidate region of chromosome 2 for T2D that includes the IL-1 family. However, current results from genome-wide studies have provided only limited support to the hypothesis that IL-1 variants affect glucose homeostasis.

This thesis describes an investigation into the role of IL-1 gene variation and IL-1 receptor antagonist in immunological responses, metabolic dysregulation and T2D. The association of common IL-1 variation with glucose homeostasis traits was studied in a population sample and replication for the findings was sought. Additionally, the genetic and nongenetic predictors of the IL-1Ra phenotype were studied in 3 independent populations. The genetic variation at the IL-1 locus was further studied as a potential predictor for T2D in two observational population cohorts. Furthermore, the systemic IL-1Ra levels were studied as potential predictors for the development of diabetes in subjects with metabolic syndrome.
2. REVIEW OF THE LITERATURE
2.1 INTERLEUKIN-1 FAMILY

2.1.1 Interleukin-1 (IL-1) alpha, beta and receptor antagonist

2.1.1.1 Characterization of IL-1 alpha, beta and receptor antagonist

The classical IL-1 family is composed of three major proteins namely IL-1 alpha (IL-1α), IL-1 beta (IL-1β) and IL-1 receptor antagonist (IL-1Ra). The ancestral IL-1 originated approximately 350 million years ago when one form of the IL-1Ra evolved as a β-stranded structure with a signal peptide. Later on, two biologically active forms of the IL-1 molecule evolved (Eisenberg et al. 1991). An analysis of sequence comparisons and mutation rates together with comparisons of the intron-exon organization of the human, mouse and rat genes indicated that gene duplication events were important in the creation of this gene family. The time line of the discoveries is reviewed by Dinarello before and after cloning of IL-1 (Dinarello (A) 2010). Two complementary DNAs (cDNAs) code for murine IL-1 and human monocyte IL-1 precursor protein (Auron et al. 1984, Lomedico et al. 1984). The NH₂-terminal portion of natural 17 kDa IL-1β originated from a precursor protein starting at amino acid 117 (Van Damme et al. 1985). The cloning, sequencing and expression of two distinct human IL-1 cDNAs eventually led to the detection of two proteins, named neutral IL-1β and acidic IL-1α (March et al. 1985). The third major protein for the IL-1 family is interleukin-1 receptor antagonist (IL-1Ra) after studies of molecular cloning, purification, expression and biological characterization (Eisenberg et al. 1990, Hannum et al. 1990, Carter et al. 1990). IL-1Ra antagonizes the biological activities of IL-1α and IL-1β (Dayer-Metroz et al.1989, Arend et al. 1989, Arend and Dayer 1990).

2.1.1.2 IL-1 alpha (IL-1α)

Stimulated human blood monocytes do not readily secrete mature IL-1α, which generally associates with the plasma membrane of the cell, exerting mainly local influences (Dinarello 2009). IL-1α is not commonly found in the circulation or in body fluids, except that the cytokine can be released from dying cells (Chen et al. 2007). Non-immune cells can also constitutively express IL-1α protein, where it is found to be critical for immunoregulatory and antiviral activity mediated by gamma interferon (IFNγ) requiring nuclear factor-κB (NF-κB) activation (Hurgin et al. 2007). The precursor form of IL-1α was
active and possibly involved in normal cellular differentiation and regulation, while the membrane associated precursor IL-1α was present in monocytes and B lymphocytes (Dinarello 2009). Calpain, a calcium-activated cysteine protease that is associated with the plasma membrane, is able to cleave the precursor into a mature molecule (Sims and Smith 2010). Most cell lines contain constitutive levels of IL-1α, which is widely expressed in various cell types such as keratinocytes and endothelial cells (Sims and Smith 2010). The patterns of IL-1α and IL-1β expression in organs vary (Hacham et al. 2002). IL-1α is expressed in lymphoreticular organs such as lungs, intestine, spleen and liver while IL-1β is expressed mostly in heart, brain, skeletal muscle and kidney. Evidence for intracellular IL-1α affecting NF-κB without activation of IL-1 receptors in non-stimulated cells has been published (Werman et al. 2004).

2.1.1.3 IL-1 beta (IL-1β)

Monocytes, tissue macrophages, and dendritic cells are considered to be the primary sources of IL-1β, which is also produced by B lymphocytes (Dinarello 2009). Nearly all microbial products can induce IL-1β expression via Toll-like receptor (TLR) signalling. IL-1β has also been shown to induce itself both in vivo and in monocytes in vitro. Whilst non-TLR ligands such as activated blood coagulation, complement activation and hypoxia also induce the synthesis of IL-1β mRNA in monocytic cells without significant translation into the IL-1β protein (Dinarello 2009). After lipopolysaccharide (LPS) stimulation, IL-1β mRNA levels increase rapidly, within minutes, and decline after 4 hours (Greten et al. 2007). The associated secretion of IL-1β from monocyte-macrophage cells increases up to 12 hours (Schindler et al. 1990, Greten et al. 2007). The subsequent downregulation of IL-1β secretion and mRNA production was more pronounced in IL-1β induced IL-1β production as compared to LPS stimulation although after stimulation with the IL-1β itself the secretion continues to rise even after 24 hours in monocytes (Schindler et al. 1990). In the circulation, IL-1β binds to large proteins like α-2-macroglobulin, complement and soluble IL-1 receptor type II (IL-1RII) (Dinarello 1996). Inactive IL-1β is cleaved by caspase-1 and active mature 18 kDa form of IL-1β is formed (Dinarello 2009). Non-caspase-1 enzymes generating active forms of IL-1β have also been described.
IL-1β induces gene expression in a variety of genes including the IL-1 family itself while the expression and synthesis of cyclo-oxygenase type 2 (COX-2), type 2 phospholipase A, and inducible nitric oxide synthase (iNOS) accounting for prostaglandin-E2 (PGE2), platelet activating factor, and nitric oxide (NO) production have been shown (Dinarello 1996). The proinflammatory activation has resulted in fever, lowered pain threshold, vasodilatation, and hypotension. Another proinflammatory property of IL-1β is its ability to increase the expression of adhesion molecules, like intercellular adhesion molecule-1, on mesenchymal cells and vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells together with the induction of chemokines. These properties of IL-1β promote the infiltration of inflammatory cells from the circulation into the extravascular space (Dinarello 1996, Libby 2002). Additionally, IL-1 promotes vascular smooth muscle cell derived cytokine production (Loppnow et al. 1998). IL-1α contributes to angiogenesis under hypoxia whereas IL-1β is found to be an angiogenic factor. IL-1β does not directly activate endothelial cell migration, proliferation and organization into blood vessel-like structures, but rather activates infiltrating cells to produce endothelial cell activating factors, such as VEGF (Carmi et al. 2009). Further studies have also supported the concept that IL-1β is released by engaging multiple distinct secretory mechanisms in human monocytes, in response to which receptors are activated (Ward et al. 2010).

In the clinical setting, intravenous injection with low doses of IL-1β from 1 to 10 ng/kg has been found to induce fever and increased levels of ACTH, blood neutrophils, nitric oxide, acute-phase proteins, several cytokines and chemokines. It has been shown that people receiving IL-1β doses of 100 ng/kg mostly become hypotensive (Dinarello 1996). Furthermore, subjects receiving doses between 30 and 100 ng/kg have demonstrated a sharp increase in cortisol and fall of glucose levels within the first hour of administration. IL-1β has also been implicated as a regulator of bone marrow hematopoietic stem cells and progenitor cells. Leucocyte counts have been shown to be increase with doses of 1 or 2 ng/kg. A rapid increase in neutrophils is associated with an increase in cortisol and circulating granulocyte-colony stimulating factor (G-CSF); levels of thyroid stimulating hormones increase, but testosterone levels decrease. IL-1α and IL-1β are known as endogenous pyrogens and also confer central nervous system mediated effects on behavior, autonomic regulation, metabolic rate, and the neuroendocrine axes (Turnbull and Rivier
IL-1 agonists cause upregulation of catecholamines, the adrenal axis and posterior pituitary hormone secretion. Conversely, gonadal and thyroidal axes are downregulated whilst the somatotropic axis and prolactin secretion are either upregulated or downregulated.

2.1.1.4 IL-1 receptor antagonist (IL-1Ra)

The IL-1 receptor antagonist gene (*IL1RN*) is the only known member of the IL-1 family to encode a cytokine with a classical signal peptide enabling secretion of IL-1Ra from the endoplasmic reticulum and Golgi apparatus (Sims and Smith 2010). IL-1Ra production is induced by a number of other cytokines, viral products, and acute phase proteins, indicating that this cytokine is produced in numerous chronic inflammatory and infectious diseases (Arend 2002). The substances that induce IL-1Ra *in vitro* are outlined in Table 1, the most potent of which include adherent IgG, LPS, GM-CSF, and IL-4 (Table 1). Glucocorticoids inhibit LPS-induced IL-1Ra and IL-1β production in monocytes, while enhanced intracellular IL-1Ra (icIL-1Ra) synthesis is seen in keratinocytes and IL-1Ra production is increased in hepatic cells (Sauer et al. 1996, Gabay et al. 1997, Arend et al. 1998). Indeed, IL-1Ra levels were elevated in the circulation of patients with a variety of inflammatory, infectious, and post-surgical conditions, indicating the importance of hepatic production of this anti-inflammatory protein (Arend et al. 1998). The balance between IL-1Ra and proinflammatory IL-1 levels has the potential for pathophysiological influences in local tissues, although the spare receptor effect, 100-fold or greater levels of IL-1Ra versus IL-1, was necessary to functionally inhibit the biologic effects of IL-1β on target cells (Gabay et al. 1997, Arend 2002). A recent clinical study has shown that upon injection of LPS into normal volunteers, the plasma concentrations of IL-1β peaked to approximately 80 pg/ml after 2 hours followed by maximum IL-1Ra concentrations of approximately 6 400 pg/ml after 3 to 6 hours.

IL-1Ra was originally described as a 17 kDa protein and its glycosylated variants of 22 – 25 kDa, also termed “soluble IL-1Ra”, are secreted from monocytes, macrophages, neutrophils, and other cells. The structural 22 kDa variant binds to both IL-1 receptors, with near equal avidity to IL-1, but does not stimulate NF-κB activation (Arend et al. 1998). The binding of IL-1Ra to IL-1RI impairs the affinity of the IL-1 receptor accessory protein (IL-1RAcP) to bind with the ligand-receptor complex. At least three
additional intracellular isoforms (icIL-1Ra) of 15, 18 and 25 kDa have been described, some of which have been found in keratinocytes, other epithelial cells, monocytes, tissue macrophages, fibroblasts, neutrophils, hepatocytes and endothelial cells (Arend 2002). It is unlikely that IcIL-1Ra inhibits intracellular binding of IL-1α to nuclear receptors in addition to its effects of receptor antagonism.

Table 1. Inducers of IL-1Ra production in human monocytes (Arend et al. 1998).

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Other inducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>Adherent IgG</td>
</tr>
<tr>
<td>M-CSF</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Acute phase proteins</td>
</tr>
<tr>
<td>IL-1</td>
<td>Products of human CMV early genes</td>
</tr>
<tr>
<td>IL-2</td>
<td>Soluble CD23 (with IL-1)</td>
</tr>
<tr>
<td>IL-3</td>
<td>IgA</td>
</tr>
<tr>
<td>IL-4</td>
<td>IgD</td>
</tr>
<tr>
<td>IL-6</td>
<td>Human Th2 cells (direct contact)</td>
</tr>
<tr>
<td>IL-10</td>
<td>Fibroneectin</td>
</tr>
<tr>
<td>IL-13</td>
<td>β-glucan</td>
</tr>
<tr>
<td>TGF-β</td>
<td></td>
</tr>
<tr>
<td>IFN-α</td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td></td>
</tr>
</tbody>
</table>

2.1.2 Genetics of the IL-1 family

2.1.2.1 Genetic structure of the IL-1 locus

Assembled collections of human genome sequences have revealed the structure of the locus on human chromosome 2 q13 that contains the 9 members of the IL-1 family (Nicklin et al. 2002, Taylor et al. 2002). The genes encoding IL-1 alpha (IL1A), IL-1 beta (IL1B) and IL-1 receptor antagonist (IL1RN) were located within a 430-kb segment of human genomic DNA, with IL1A being the most centromeric and IL1RN the most telomeric. The other six
IL-1 genes in this region all are located between *IL1B* and *IL1RN*. In general, the organization of the mouse *IL-1* locus is similar to that of the human locus (Taylor et al. 2002). Three common exon sequences, related to the known β-sheet structure, have been defined for *IL1B* and *IL1RN* (Nicklin et al. 2002). The gene order *IL1A - IL1B - IL1F7 - IL1F9 - IL1F6 - IL1F8 - IL1F5 - IL1F10 - IL1RN* is related to the evolutionary relationships between the genes. Phylogenetic analyses suggest that the earliest evolutionary event in the diversification of *IL-1* was the bifurcation of *IL18* from the *IL-1* precursors. This was followed by formation of *IL1A* from *IL1B/IL1RN*, which later developed into the *IL1B* gene and other members of the *IL-1* family (Nicklin et al. 2002).

2.1.2.2 Common *IL-1* variation

Determination of linkage disequilibrium (LD) in *IL-1* genes has verified a reasonable correlation between disequilibrium and the physical distance of the markers (Cox et al. 1998). The structural genetic variation in the *IL-1* gene region has been derived from the Seattle single nucleotide polymorphism (SeattleSNP) resequencing data, including the haplotype tagging variants (National Heart, Lung, and Blood Institute, SeattleSNPs. Program for Genomic Applications. Seattle, WA: SeattleSNPs. pga.gs.washington.edu). At least 450 common variant SNPs in *IL-1* genes have been identified covering the haplotypic variation with a correlation coefficient ($r^2$) of more than 0.8 based on HapMap data (Phase III, National Center for Biotechnology Information B36 assembly).

The analysis of the *IL1B* promoter region demonstrates the importance of the population specific haplotype in the context of the functionally significant regulatory regions. Single SNPs may exert either down- or up-regulatory effects whilst being at the same time in near complete LD with each other (Chen et al. 2006). Evolutionary haplotype structures of *IL1RN* confer four major haplo-groups (clades) based on SNPs and variable number tandem repeat (VNTR) polymorphisms. Three clades associated with the common *IL1RN* VNTR, with four copies of 86-bp repeats in the Seattle SNPs data, and one clade were associated with allele 2 of *IL1RN* VNTR (IL1RN*2) consisting of two copies of 86-bp repeats (Reiner et al. 2008). Finally, there are six different *IL1RN* VNTR polymorphisms of which the two most common account for over 95% of the cases (Vamvakopoulos et al. 2002).
Danis and coworkers reported that IL1RN*2 is associated with increased IL-1Ra production and decreased cellular IL-1α in cultured monocytes that were isolated from healthy blood donors after stimulation with a panel of cytokines (Danis et al. 1995). Another small study reported increased IL-1Ra production or IL-1Ra/IL-1β ratio in IL1RN*2 carriers after stimulation of monocyctic cells with specific pathogenic microbial antigens (Wilkinson et al. 1999). Plasma levels of IL-1Ra were also increased in IL1RN*2 carriers and co-ordinately regulated with the promoter and coding IL1B variants (Hurme and Santtila 1998). Concordantly, a cross-sectional study of young non-obese men found that IL-1Ra levels were increased in the presence of IL1RN*2, which exerts an allele dose-dependent association (Strandberg et al. 2006). Accordingly, European reports describe individuals carrying one IL1RN SNP in complete LD with IL1RN*2, which was found to associate in a dose-dependent manner with increased IL-1Ra production from blood cells after peptidoglycan stimulation (Reiner et al. 2008). Additionally, another IL1RN SNP rs4251961 originating from a different haplotypic group, or clade, was even more significantly associated with decreased IL-1Ra levels (Reiner et al. 2008). Recent studies of the genetic variation in the IL-1 family have produced genome-wide evidence that the IL1RN variants are associated with systemic IL-1Ra levels, and a quantitative trait locus was reported (Melzer et al. 2008). Genotyping of seven common SNPs (minor allele frequencies > 0.1) by using HapMap phase II NCBI B34 assembly capturing 69 % of the SNPs across IL1RN ($r^2$ threshold $\geq 0.8$) revealed a robust association of rs4251961 with decreased IL-1Ra levels (Rafic et al. 2007). One of the IL1RN*2 tagging variants was independently associated with increased IL-1Ra levels (Rafic et al. 2007).

Studies of gene to gene product association with IL1B variation suggested that multiple variants in the coding and promoter region are functionally significant (Sandtilla et al. 1998, Hall et al. 2004, Iacoviello et al. 2005). Genome wide association studies (GWAS) have also been able to identify a significant association between IL-1β mRNA level and an IL1B promoter variant that was in strong LD with rs1143634 (Melzer et al. 2008). The coding IL1B variant was suggested to have associations with increased CRP levels in the studies of healthy blood donors and patients with coronary heart disease (Latkovskis et al. 2004, Carter et al. 2008). Common IL1B variation of ten haplotype tagging SNPs ($r^2 > 0.64$) has also been related to the change of CRP among obese young adults examined in
the Coronary Artery Risk Development in Young Adults (CARDIA) study and the change in CRP levels was analyzed in relation to baseline after 8 years (Enquobahrie et al. 2009). The results suggested a weak to modest association between the IL1B genetic variation and change in CRP levels among obese young adults. Obesity was found to modify the association of IL1B haplotypes with CRP among blacks and whites in different ways. Variation in the transcriptional regulatory region of the IL1A gene has shown evidence of association with IL-1α mRNA and protein production with the promoter variation (Dominici et al. 2002).

2.1.2.3 Inherited human syndromes of IL-1 genes and IL-1β production

The concept that severity of inflammation has been influenced by the relative amounts of IL-1 cytokines and IL-1Ra gained new ground after two reports of 10 infants with germ-line mutations in the IL-1 family genes (Aksentijevich et al. 2009, Reddy et al. 2009). An autosomal recessive autoinflammatory syndrome was described around birth with multifocal osteomyelitis, periostitis, and pustulosis including homozygous truncating single point mutations in IL1RN gene, in addition to a large deletion in chromosome 2q in two cases lacking IL1RN together with five other genes of the IL-1 family. Functional studies revealed a striking alteration in IL1RN expression and IL-1Ra secretion together with increased IL-1-dependent inflammatory activity. Nearly all cases had bone abnormalities consisting of numerous osteoclasts, osteolytic lesions, and sterile osteomyelitis. Additionally, high levels of inflammatory markers, thrombocytosis, neutrophilia and inflammation related multiorgan failure was reported. Infants with IL-1Ra deficiency were treated with recombinant IL-1 receptor antagonist (anakinra) which resulted in a rapid reduction in inflammation and skin eruptions; with prolonged treatment, bone abnormalities regress and children developed normally.

A number of autoinflammatory diseases have been described as having specific mutations in proteins regulating IL-1β or other known factors responsible for the blockade of IL-1β activity (Dinarello 2009). The discovery of nucleotide binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3) mutations in human disease was characterized by recurrent fever and inflammation comprising three disorders (Schroder and Tschopp 2010). The diseases described in order of increasing severity were
familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and chronic infantile cutaneous neurological articular syndrome (CINCA; also called neonatal-onset multisystem inflammatory disease, NOMID). Myeloid cells from these patients were hyper responsive for IL-1β production and patients suffering from these syndromes responded well to IL-1β antagonism with anakinra. Both early diagnosis and therapy initiation were required for effective long-term treatment (Neven et al. 2010).

2.1.2.4 IL-1 family knockout phenotypes

Genetic deletion of the IL-1 family members exerts marked effects in various brain tissues (Horai et al. 1998). IL-1α mRNA levels are reduced 30-fold in the IL-1β knock-out mouse model after induction with turpentine as compared to wild type mice. Alternatively, IL-1β mRNA is reduced 5 to 10-fold in the IL-1α knock-out mice. IL-1α induction in the brain and febrile responses are markedly dependent on the presence of IL-1β. Corticosteroid levels in the serum of mice deficient for IL-1α/β are also lower 8 hours after turpentine injection as compared to wild type mice.

Mice lacking the IL1RN gene suffer growth retardation in adult life and IL-1Ra is required for attaining normal body mass (Hirsch et al. 1996, Horai et al. 1998, Sims and Smith 2010). Deficiencies in IL-1β or IL-1α do not cause spontaneous disease in the unchallenged knockout model, but deficiency of IL-1β was found to associate with decreased acute phase responses. Genetic depletion of IL1A, IL1B or IL1RI has been related to increased severity of various infections and reduced atherosclerotic lesion size, while IL1RI depletion is associated with mature-onset obesity and maintenance of insulin sensitivity in obese mice on a high-fat diet (Sims and Smith 2010).

2.1.3 The IL-1 receptor family

Nearly all IL-1 receptor (IL1R) family members belong to the group containing three immunoglobulin (Ig)-like binding domains in the extracellular receptor domain, however some remained orphan receptors (Table 2). IL-1RI, IL-1RII and IL-1R accessory protein (IL-1RAcP) form the most important group of receptors. Receptors IL-1R8 and IL-1R9 were found on the X-chromosome while a single Ig-like domain SIGIRR receptor has been
described as a negative regulator of both IL-1α and IL-1β activities while acting also as TLR agonists in tandem (Dinarello 2009).

Table 2. The IL-1 receptor family (Dinarello 2009).

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligands</th>
<th>Coreceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1RI</td>
<td>IL-1α, IL-1β, IL-1Ra</td>
<td>IL-1RAcP (IL-1R3)</td>
</tr>
<tr>
<td>IL-1RII</td>
<td>IL-1β, IL-1β precursor</td>
<td>IL-1RAcP (IL-1R3)</td>
</tr>
<tr>
<td>ST2/Fit-1</td>
<td>IL-33</td>
<td>IL-1RAcP (IL-1R3)</td>
</tr>
<tr>
<td>IL-18Rα</td>
<td>IL-18, IL-1F7</td>
<td>IL-18Rβ (IL-1R7)</td>
</tr>
<tr>
<td>IL-1Rrp-2</td>
<td>IL-1F6, IL-1F8, IL-1F9</td>
<td>IL-1RAcP (IL-1R3)</td>
</tr>
<tr>
<td>TIGIRR-2/IL-1RAPL</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>TIGIRR-1</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>SIGIRR</td>
<td>unknown</td>
<td>unknown</td>
</tr>
</tbody>
</table>

The IL-1RI receptor has a high level of homology with the cytoplasmic domains of Toll-like receptors (Dinarello 2009). The cytoplasmic domain of IL-1RI includes a binding domain for the myeloid differentiation primary response gene 88 (MyD88) protein. The signalling cascade includes production of Iκ kinase beta (IKKβ) and phosphorylated Iκβ degrades releasing NF-κB which, in turn, enters the nucleus (Akira et al. 2001, Dinarello 2009). Mitogen activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) are also activated.

2.1.4 The IL-1 family of ligands

Currently known members of IL-1 family are shown in Table 3. All the genes encoding IL-1 family members have been located in a 400 kb region of human chromosome 2 except IL-18 and IL-33 (Sims and Smith 2010). Caspase-1 is needed to produce mature IL-18 that is expressed by macrophages, dendritic cells (DCs) and epithelial cells. IL-18 has typically been described as a T helper cell type 1 promoting cytokine. Several components are involved in MyD88-independent signalling pathways that are responsible for interferon
gamma (IFNγ) gene expression, caspase activation and costimulatory molecule induction, including IL-18 that also leads to IFNγ inducible gene expression (Akira et al. 2001). A recent case-control study of subjects with type 2 diabetes suggested that IL-18 may be a predictor for the progression of diabetic nephropathy or cardiovascular disease (Nakamura et al. 2005). The recently discovered IL-33 is expressed in many tissues, is not processed by caspase-1, and acts as a transcriptional repressor by promoting the compaction of chromatin (Sims and Smith 2010). IL-33 is also associated with the differentiation of T helper cells.

Table 3. IL-1 family members (Dinarello 2009).

<table>
<thead>
<tr>
<th>New Name</th>
<th>Traditional Name</th>
<th>Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1F1</td>
<td>IL-1α</td>
<td>Agonist</td>
</tr>
<tr>
<td>IL-1F2</td>
<td>IL-1β</td>
<td>Agonist</td>
</tr>
<tr>
<td>IL-1F3</td>
<td>IL-1Ra</td>
<td>Receptor antagonist</td>
</tr>
<tr>
<td>IL-1F4</td>
<td>IL-18;IFN-γ-inducing factor</td>
<td>Agonist</td>
</tr>
<tr>
<td>IL-1F5</td>
<td>FIL1δ</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>IL-1F6</td>
<td>FIL-1ε</td>
<td>Agonist</td>
</tr>
<tr>
<td>IL-1F7</td>
<td>IL-1H4, IL-1ζ</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>IL-1F8</td>
<td>IL-1H2</td>
<td>Agonist</td>
</tr>
<tr>
<td>IL-1F9</td>
<td>IL-1ε</td>
<td>Agonist</td>
</tr>
<tr>
<td>IL-1F10</td>
<td>IL-1Hy2</td>
<td>Receptor antagonist (?)</td>
</tr>
<tr>
<td>IL-1F11</td>
<td>IL-33</td>
<td>Agonist</td>
</tr>
</tbody>
</table>

2.1.5 Inflammasome activation

The term ‘inflammasome’ is used to describe a large (700 kDa), multiprotein complex that is formed under certain conditions in vitro and is sufficient to trigger the activation of caspase-1 (Martinon et al. 2002). Caspases are a family of cysteine proteases with an important role in mammalian apoptosis or proteolytical activation of cytokines. They generally recognize a tetrapeptidic sequence having specificity for cleaving the peptide bond C-terminal to aspartic acid residues (Martinon and Tschopp 2004). The cytoplasmic protein, nucleotide binding oligomerization domain-like (NOD-like) receptor (NLR),
mediates innate immune responses belonging to pattern recognition receptors (PRRs) (Martinon et al. 2009). NLRs form central molecular platforms that organize signalling complexes such as inflammasomes, where the activation is triggered by adenosine triphosphate (ATP), allergens, products of dying cells and uric acid. Although ATP is emerging as an important modulator of inflammation, the critical role of extracellular ATP as a danger signal remains unclear (Martinon et al. 2009). Caspase-1, also known as interleukin-1 converting enzyme (ICE), belongs to the subgroup of inflammatory caspases that is responsible for processing the maturation of IL-1β.

The precursor form of IL-1β is known to accumulate in the cytoplasm, and activation of caspase-1 is triggered by ATP activating the purinoreceptor (P2X7) (Figure 1). The loss of intracellular K+ induced by P2X7 activation appears to be crucial for the acceleration of caspase-1 processing (Kahlenberg and Dubyak 2004). It has been postulated that less than physiological amounts of potassium are required for spontaneous inflammasome formation suggesting that the inflammasome may sense drops in potassium levels. Various danger signals and stimuli that activate the NALP3 inflammasome are able to trigger potassium efflux, thereby lowering the cytosolic potassium concentration of stimulated cells (Martinon et al. 2009). Coinheritance of twelve functionally relevant P2X7 SNPs shows associations with IL-1β secretion from stimulated monocytes suggesting that the haplotypic variation plays an important role in genetic association studies of several disorders related to inflammation (Stokes et al. 2010).

Monocyte-macrophage cells are considered to be effectors of innate immunity. Monocytes originate from bone marrow and circulate with a half-life from 1 to 3 days. Tissue macrophages arise from monocytes that have migrated from the circulation, or were formed by in situ proliferation of their local precursors. The synthesis and release of IL-1β is different between human monocytes and macrophages (Ward et al. 2010). For example, monocytes confer ATP (P2X7 receptor) independent IL-1β release while the release in macrophages is dependent on ATP. Monocytes are constitutively seen with activated caspase-1, leading to the release of active IL-1β after LPS stimulation, whereas macrophages require two distinct stimuli: one stimulus induced transcription and translation, and a second stimulus is needed for caspase-1 activation with subsequent IL-1β processing and secretion requiring ATP (Netea et al. 2008). Recent studies have also
revealed a possible P2X7 receptor-independent release of mature IL-1β from monocytes, distinctly from macrophages (Ward et al. 2010).

Externalization of mature IL-1β and caspase-1 together with lysosomal proteins is facilitated by extracellular ATP triggering the efflux of K+ from cells, followed by Ca^{2+} influx and activation of three phospholipases: phosphatidylcholine-specific phospholipase C and calcium-independent and -dependent phospholipase A2 (Andrei et al. 2004). Different models have been described explaining the rapid release of mature IL-1β, including exocytosis of secretory lysosomes, shedding of plasma membrane microvesicles, and direct efflux through plasma membrane transporters, as well as an alternative model of formation of multivesicular bodies that contain exosomes (Qu et al. 2007).
Figure 1. Steps in the synthesis and secretion of IL-1β induced by IL-1β (modified from Dinarello 2009). Primary blood monocytes or tissue macrophages are activated by IL-1β (step 1). The formation of the IL-1 receptor complex heterodimer results in approximation of the Toll IL-1 receptor (TIR) domains and the recruitment of MyD88. The transcription (step 2) and translation into the IL-1β precursor (step 3) are separate events. Translation takes place in the cytosol, not in the endoplasmic reticulum. The activated monocyte/macrophage releases ATP into the extracellular space. Upon activation of the P2X7 receptor by ATP (step 4), there is a rapid efflux of potassium from the cell (step 5a) resulting in a fall in intracellular levels of potassium (step 5b). The fall in intracellular potassium levels triggers the assembly of the components of the NALP3 inflammasome (step 6). Active caspase-1 processes the IL-1β precursor (step 7) in the cytosol or in the secretory lysosome, resulting in the generation of the carboxy-terminal mature IL-1β. An influx of calcium into the cell (step 8) with an increase in intracellular calcium levels provides a mechanism by which mature IL-1β is released from the cell (step 9). The rise in intracellular calcium activates phosphatidylycholine-specific phospholipase C and calcium-dependent phospholipase A(2), which facilitate the secretion of IL-1β (step 9) with exocytosis of the lysosomal contents.
2.1.6 Regulation in IL-1 activation

2.1.6.1 Regulation at receptor and nuclear level

IL-1 cytokines are known as upstream mediators of inflammatory responses in local cellular interactions and regulators for hepatic acute phase protein production. Because of their potency and extensive functions, the biological activity of IL-1α and IL-1β is tightly regulated. IL-1α and IL-1β are expressed at low levels under normal conditions and require induction at both transcriptional and translational levels (Sims and Smith 2010). Unlike other cytokine families, the IL-1 family exerts its control over inflammation at both receptor and nuclear levels (Dinarello 2009). IL-1Ra has been shown to bind with high affinity to IL-1RI and the recruitment of IL-1RACP is inhibited by differential binding properties to IL-1 agonists (Arend et al. 1998). At the same time, IL-1Ra blocks the binding of IL-1α and IL-1β to IL-1RI. Other regulators of IL-1 activity include the IL-1 type II decoy receptor (IL-1RII), which mainly prevents excessive autocrine activation of IL-1 signalling while binding of IL-1Ra to IL-1RII is altered (Mantovani et al. 1998, Sims and Smith 2010). In addition to IL-1Ra the single Ig IL-1-related receptor (SIGIRR) is a suppressor of inflammation, and the IL-18-binding protein (IL-18BP) has been found to be the high-affinity endogenous neutralizer for IL-18 activity (Dinarello 2009).

Several proteins have been suggested to have regulatory roles for IL-1β activity by interfering with the activity of caspase-1, while the processing of pro-IL-1β in macrophages is negatively regulated by NF-κB-dependent gene products (Martinon and Tschopp 2004, Greten et al. 2007). Micro RNAs (miRNAs) have been shown to play a role in modulating the IL-1 pathway in human dendritic cells (DCs) by directly targeting adaptor molecules of the TLR/IL-1 signalling cascade; miRNAs are up-regulated upon LPS stimulation of human primary dendritic cells (Ceppi et al. 2009). IL-1 is proposed to be an activator for dendritic cells, although the role of IL-1 proteins in unique biological functions in dendritic cells has not been resolved (Blanco et al. 2008).

2.1.6.2 IL-1 family and T cell polarization

The role of IL-1α or IL-1β as a costimulator of T cell functions has been described, primarily together with an antigen or a mitogen (Dinarello 2009). As for the early pro-inflammatory response, DC activation is triggered by pattern recognition receptor signals
and these signals promote the expansion and differentiation of naive pathogen-specific T cells (Joffre et al. 2009). IL-1β has been shown to induce the expansion of naive and memory CD4+ antigens and the responses are prevented by IL-1Ra (Ben-Sasson et al. 2009).

Studies into airway hypersensitivity responses in mice lacking IL-1α/β or IL-1Ra genes have demonstrated that specific T helper cell type 2 (Th2) activation requires IL-1 cytokines (Nakae et al. 2003). Cytokines IL-1β and IL-1α are also required for Th2 specific cytokine profiles, adhesion molecule expression, and inflammation, but neutralization of IL-1α alone does not prevent a hypersensitivity reaction (Johnson et al. 2005). IL-1β has been found to strongly increase B cell proliferation in the presence of a mitogenic stimulus like surface immunoglobulin or costimulatory molecule (Sims and Smith 2010). T cell dependent antibody production is enhanced in mice lacking IL1RN as determined by IgG production (Nakae et al. 2001). However, an excess IL-1 signalling in IL-1Ra deficiency alone did not enhance IgM production. Additionally, mice deficient of IL-1α/β or IL-1β showed reduced IgG and IgM production.

T helper cell type 17 (Th17) has been associated with experimental autoimmune diseases where IL-1β and TNFα were independently shown to promote Th17 responses in IL-1RI deficient mice (Sutton et al. 2006). IL-1β is proposed to play a key role in the differentiation of Th17 cells from naïve precursors (Sims and Smith 2010). In human studies, TGFβ together with IL-6 contribute to the differentiation of Th17 cells and a similar costimulatory function for IL-1β has been shown for the development of Th17 cells (Korn et al. 2009). IL-1β and IL-6 have been found to be promoters and maintainers for the differentiation of human naïve CD4+ T cells into Th17 cells.

2.1.7 Pathological and physiological properties of the IL-1 family

2.1.7.1 Diabetes and metabolic traits

Metabolic regulation at peripheral level has been studied using mouse models lacking IL-1α/β and IL-1Ra (Matsuki et al. 2003). Mice deficient for IL-1Ra were lean with a metabolic dysregulation caused by excess IL-1 signalling or via central mechanisms. Importantly, the insulin levels were low and no significant change was seen in glucose levels in the feeding experiments. In this context decreased insulin concentration together
with IL-1Ra depletion may have caused reduced fat accumulation in adipose tissue and excess IL-1 signalling that altered lipoprotein lipase activity resulting in suppression of fat accumulation. Increased hypothalamic IL-1β mRNA expression is found with glucocorticoid deficiency following adrenalectomy while deficient leptin signalling is associated with lower IL-1β expression suggesting an important role in energy homeostasis (Wisse et al. 2004).

The connection between the IL-1 family and diabetes was initially shown with the deleterious effects of IL-1β on pancreatic beta cells and increased IL-1Ra levels in insulin dependent diabetes (Mandrup-Poulsen et al. 1986, Perrier et al. 2006). Elevated levels of IL-1Ra are suggested to be protective for beta-cell destruction or dysfunction. Recent studies have also developed hypotheses for the role of the IL-1 family in diabetes affected by the IL-1β activity versus IL-1Ra levels determining the outcome of islet inflammation in T2D (Dinarello (B) et al. 2010).

IL-1 cytokines affect glucose homeostasis in pancreatic beta cells, adipose tissue and at central levels. Elevated concentrations of glucose are able to induce IL-1β production and secretion in human beta cells, leading to Fas receptor up-regulation, NF-κB activation, beta cell apoptosis, and dysfunction (Maedler et al. 2002). Glucose and IL-1β induced deterioration of beta cells is also dependent on Ca++ influx and extracellular signal regulated kinase activation (ERK) (Maedler (A) et al. 2004). Glucose or IL-1β stimulation is followed by increased expression of IL-1β mRNA in purified beta cells. The basal mRNA levels, however, are lower in the cases with higher IL-1β expression following glucose stimulation (Boni-Schnetzler et al. 2008). Chronic exposure to increased IL-1β levels has been shown to lead to inhibited insulin signal transduction and altered adipose tissue lipid content and differentiation (Lagathu et al. 2006, Jager et al. 2007). IL-1β is therefore capable of inducing insulin resistance by inhibiting insulin receptor substrate -1 (IRS-1) expression mainly through messenger RNA, the amount of which is dependent on the extracellular receptor (regulated) kinase pathway (ERK), and also by a posttranscriptional mechanism independent of the extracellular receptor kinase. In acute inflammation, the short-term stimulation of IL-1α leads to transient insulin resistance in adipocytes as shown by altered insulin signalling phosphorylations of IRS-1 (He et al. 2006). Central or peripheral administration of IL-1β is capable of inducing rapid glucose
uptake by cells followed by counter regulatory responses of increased glucagon, catecholamines, and glucocorticoids to maintain glucose homeostasis (del Rey et al. 1998, del Rey et al. 2006).

Increased expression and secretion of the natural IL-1Ra from adipose tissue has previously been associated with obesity (Juge-Aubry et al. 2003). The levels of plasma IL-1Ra were increased nearly 7-fold in morbidly obese patients when compared with lean control subjects, and their levels of insulin resistance assessed by a homeostasis model correlated with IL-1Ra levels (Meier et al. 2002). In the same study, surgical intervention for obesity was followed by a significant decrease in cytokine antagonist levels. Cross-sectional studies have found elevated IL-1Ra levels in obese subjects with impaired glucose tolerance and metabolic dysregulation, although in patients with suspected coronary artery disease the IL-1Ra levels were decreased in T2D as compared with nondiabetic individuals (Marculescu et al. 2002, Ruotsalainen et al. 2006). In a prospective case-control study of Whitehall II cohort, elevated systemic levels of IL-1Ra predicted the incidence of type 2 diabetes mellitus (Herder et al. 2009). An attenuation of the association was observed after adjustment for 2 hour glucose and waist circumference. A similar finding was observed in a prospective follow-up study of the FINRISK 97 cohort and Health 2000 cohort in Finland (Salomaa et al. 2010). IL-1Ra levels have been reported to increase in an accelerated manner during the last 6 years before the diagnosis of T2D, but the measures of adiposity do not explain the serial changes in the cytokine antagonist levels (Carstensen et al. 2010).

In addition to the prognostic role of IL-1Ra in the development of T2D, the effects on glucose homeostasis are considered beneficial and protective for maintaining glucose homeostasis. In a recent clinical trial, patients with T2D were randomly assigned to receive human recombinant IL-1Ra, anakinra, for 3 months. Improved glycemia and enhanced C-peptide secretion as well as reduction in the ratio of proinsulin to insulin were found in patients receiving anakinra as compared to patients receiving placebo suggesting improved beta cell function (Larsen et al. 2007). There were no alterations in insulin sensitivity on the basis of insulin clamp studies, insulin sensitivity index after oral glucose-tolerance test and body-mass index remained stable. The relative therapeutic effects of IL-1Ra administered to mice on a high-fat diet include; improved glucose tolerance, insulin secretion, insulin sensitivity and suggested decreased serum levels of total cholesterol,
triglycerides, and free fatty acids (FFA) (Sauter et al. 2008). Alternatively, a model of impaired insulin secretion has shown that IL-1Ra treatment is protective for increased islet proinflammatory cytokine expression, chemokine expression, islet immune cell infiltration, and improved insulin processing (Ehses et al. 2007). Reduced proinsulin to insulin ratio and insulin resistance indices were associated with the treatment group while high-dose IL-1Ra treatment has had little effects on circulating insulin compared to lower dose treatment group.

Increased IL-1Ra levels have been associated with preserved beta cell capacity in recently diagnosed type 1 diabetes in young patients, supporting the beneficial role of IL-1 antagonism in glucose homeostasis (median age 9.6 years) (Pfleger et al. 2008). Whilst local IL-1 regulation may not be reflected by systemic levels of IL-1Ra, the expression of IL-1Ra mRNA in pancreatic islets is down regulated in T2D subjects while the beta cell expression and secretion of IL-1Ra are modulated by exogenous leptin and IL-1Ra (Maedler (B) et al. 2004). IL-1Ra expression and beta cell function may be regulated locally by inhibition of IL1RN expression, however exogenous IL-1Ra is protective for beta cell dysfunction and survival. Furthermore, it is suggested that IL-1Ra may lower serum lipid levels leading to improved insulin sensitivity and beta cell function, while excess IL-1Ra in circulation is followed by increased IL-1Ra expression in adipocytes (Sauter et al. 2008).

Reports concerning the association of IL-1 gene variation with T2D or related traits are controversial. The extended follow-up for 39 weeks of T2D diabetes after withdrawal of anakinra therapy demonstrated sustained effects with reduction of proinsulin to insulin ratio, CRP and IL-6 in the treatment group compared to placebo (Larsen et al. 2009). Clinical responders to anakinra displayed lower baseline IL-1Ra levels compared to nonresponders and the difference remained similar 39 weeks after withdrawal of the therapy. Additionally, the minor allele of the IL1RN SNP rs4251961, known to be associated with lower serum IL-1Ra, was significantly overrepresented in responders, suggesting that genetically controlled low endogenous IL-1Ra levels could have a predictive role in the response to anakinra while the VNTR polymorphism did not modify the response to IL-1 antagonist therapy.
A trend towards association with glucose homeostasis traits for one of the IL1RN SNPs - rs4251961 has been suggested (Rafic et al. 2007). However, a meta-analysis consisting of three cross-sectional genome-wide association studies (GWAS) was unable to show an association between IL1RN variation and T2D including variant that is in strong LD with rs4251961 (Rafiq et al. 2008). In a study of two populations of young and elderly men, an association between total fat mass and fat accumulation in different compartments was reported when metabolic traits and IL1RN variation were investigated (Strandberg et al. 2006, Andersson et al. 2009). A study of premenopausal Asian women suggested an association of IL1B coding variant rs1143634 with body mass index while another study of Caucasian individuals with coronary heart disease (CHD) with male predominance found an association between central obesity and rs1143634 (Um et al. 2004, Carter et al. 2008). The minor alleles of the promoter IL1A rs1800587 and the coding IL1B rs1143634 were associated with waist circumference values, suggesting an allele-dose relationship for the latter variant. Conflicting results for IL1B rs1143634 have been reported with young lean European men suggesting a decreased body mass index for minor allele carriers of IL1B rs1143634 and no association for the IL1B promoter variant (Strandberg et al. 2006). An association of body fat mass and IL1B promoter variation in elderly Caucasian men has also been reported, although the relationships did not behave in a linear manner with regard to allele-dose relationships (Strandberg et al. 2008).

2.1.7.2 Thyroiditis

Proinflammatory IL-1 contributes to direct local effects on thyroid cells by affecting the thyroid-specific proteins followed by down regulation of thyroid-derived proteins (Poncin 2008). IL-1β decreases the release of thyroglobulin and the effects are also counteracted by IL-1Ra (Rasmussen et al. 1997). Additionally, the release of thyroidal cyclic adenosine monophosphate (cAMP) is inhibited by IL-1β and IL-1Ra whereas IL-1β but not IL-1Ra stimulation is followed by increased cyclic guanosine monophosphate (cGMP) release. IL-1α reduces thyroid cellular integrity and barrier function, suggesting vulnerability to autoimmune responses (Nilsson et al. 1998). Accordingly, when thyroid cells are exposed to IL-1α/IFNγ, the thyroid peroxidase mRNA expression and protein levels are down regulated (Gerard et al. 2006), whilst Th2 specific IL-4 is able to directly reverse the
inhibitory effects of IL-1α/IFNγ stimulation in thyroid cell lineages (Poncin et al. 2008). Lymphocytic infiltration is known as a common feature in all forms of autoimmune thyroid diseases (AITD) (Weetman 2004), and IL-1β has been proposed to affect both thyroid follicles and T cell responses.

AITDs are one of the most common autoimmune disorders, affecting between 2 and 4% of women and up to 1% of men. Furthermore, AITD prevalence increases increasing with age and is associated with subclinical hypothyroidism (Vaidya et al. 2002). The genetic component in AITD is evident due to an increased concordance in twins, familiar aggregation of the syndromes, increased heritability of thyroid autoantibodies and associations with other autoimmune diseases (Vaidya et al. 2002).

**IL1RN** and **IL4** gene polymorphisms are proposed to have an association with AITD in Caucasian cohorts, such as with hyperthyroidism related to Graves’ disease, but the results have not been replicated in further studies (Hunt et al. 2000, Heward et al. 2001). The minor allele of **IL1A** promoter region rs1800587 is associated with Graves’ disease and related oftalmopathy, the latter of which has shown consistent findings in another study in Asian individulas (Liu et al. 2010). Additionally, the minor allele of **IL1B** promoter rs16944 has been suggested to have a protective role for Graves’ disease when compared to healthy controls in Asian population samples (Liu et al. 2010). An association between intractable Graves’ disease with positive thyroid antibodies and another **IL1B** promoter minor variant, which is in complete LD with rs16944, was proposed when the cases in remission and negative for thyroid antibodies were compared (Hayashi et al. 2009). At the same time, an increased proportion of peripheral T helper type 17 (Th17) cells are also reported to associate with the **IL1B** promoter variant.

**2.1.7.3 Cardiovascular diseases**

IL-1β is involved in the initiation and progression of atherosclerosis (Libby 2002). Different forms of cholesterol have also been reported to induce or activate IL-1 cytokines (Lin et al. 2003, Duewell et al. 2010). Cholesterol crystals, that are known to induce IL-1β release, are proposed to act as inflammatory factors in vascular injury as has also been shown for oxidised lipoproteins. Furthermore, IL-1Ra and IL-1β are present in the vascular endothelial layers and the expression of IL-1 gene products are up regulated in human

Studies of proatherogenic mice with high fat feeding suggest that genetic deletion of the IL-1 receptor type I or administration of IL-1Ra may inhibit atheroma formation and a rise in blood pressure in response to environmental stimulus (Chamberlain et al. 2009). IL-1β seems to cause neointimal thickening of the arterial wall in IL-1Ra deficiency and the proatherogenic model with IL-1Ra deficiency modulates plaque composition with increased inflammation and macrophage content (Isoda et al. 2003, Isoda et al. 2004). IL-1Ra depletion is associated with increased fat content of the liver and decreased excretion of bile acids, whilst a more than ten-fold increase in the liver IL-1β mRNA and four-fold increase in TGFβ expression is seen after four weeks of high-fat feeding (Isoda et al. 2005). Several other complex proatherogenic models also support the concept that the IL-1 family contributes to the development of atherosclerosis and interacts with cholesterol levels (Devlin et al. 2002, Kirii et al. 2003, Kamari et al. 2007).

A possible association between IL-1 variation and coronary artery disease (CAD) has been studied with conflicting results (Francis et al. 1999, Vohnout et al. 2003). A nested case-control study of US physicians aged 40 to 84 years showed no evidence for an association of variation in the IL-1 gene family with MI or stroke, including also IL1B rs16944 and IL1RN VNTR (Zee et al. 2001, Zee et al. 2008). Only rs1143623 of IL1B showed a modest association toward reduced risk of myocardial infarction (MI). Patients younger than 50 years had a suggestive association between the IL1B rs16944 minor allele and decreased number of atherothrombotic events in a case control study of MI and stroke (Iacoviello et al. 2005). At the same time the minor allele of rs16944 associated with increased tissue factor expression from lipopolysaccaride stimulated mononuclear cells. A family based association study of CAD showed consistent results with Iacoviello et. al. in subjects younger than 50 years by studying haplotypes of the three coding or promoter IL1B and IL1A variants (Brown et al. 2010). However, adjusting for hypercholesterolemia attenuated the significant associations. IL1RN haplotypes (including SNPs rs2232354, rs315952, and rs315949) have been investigated in a cohort of European MI survivors, suggesting an association with the IL-1Ra messenger RNA levels and MI (van Minkelen et al. 2009). Studies on restenosis after coronary angioplasty, however, did not show a
substantial association with IL-1 variation (Kastrati et al. 2000, Francis et al. 2001, Zee et al. 2003).

In a case-control study on carotid atherosclerosis, an association between significant stenosis (>50%) and *IL1RN* VNTR was found by multivariate analysis, with high discrimination under the receiver-operating characteristic curve and a gene-dose effect for IL1RN*2 (Worrall et al. 2003). Diabetes, hypertension and hypercholesterolemia were the predominating risk factors amongst the significant carotid atherosclerosis group and the prevalence of CAD was more than 50%. However, conflicting results were reported from studies in MI survivors using quantitative coronary angiograms suggesting a decreased plaque area in IL1RN*2 carriers (Olofsson et al. 2009). IL1RN*2 has further been analyzed in three cohorts suggesting an association between homozygote genotype and cerebrovascular stroke in white population while the results in non-whites remained inconclusive (Worrall et al. 2007). Other IL-1 variants were not associated with stroke in a nested case-control study (Zee et al. 2008).

2.1.7.4 Other disorders

The IL-1 family of proteins have been linked to a number of other clinical disorders. Increased levels of IL-1Ra are found in the cervical mucosa at the time of ovulation and decreased levels during the luteal phase of the menstrual cycle (Huang et al. 2001, Witkin et al. 2002, Arend 2002). The presence of IL-1Ra in intra-amniotic fluid stimulated by IL-1β has also been shown. IL-1 family proteins are also found in the endometrium where IL-1Ra appears to exert an inhibitory influence on embryonic implantation while elevated concentrations in amniotic fluid suggest an increased risk for preterm birth. IL1RN*2 is suggested to be protective for spontaneous abortions while the *IL1B* promoter variants are thought to have associations with recurrent pregnancy loss, further proposing a possible interaction with adaptive immune responses (Wang et al. 2002, Perni et al. 2004). The effect of IL-1Ra depletion on male fertility has been studied using an IL-1Ra knock-out mouse model where a direct correlation between high levels of IL-1 in the testes and a reduction of male mouse fertility was reported (Ganaiem et al. 2009).

A systematic meta-analyses of the common IL-1 variation in schizophrenia reported suggestive associations with the *IL1B* promoter variant (rs16944) (Allen et al.
2008, Xu and He 2010). The involvement of IL-1 and IL-1Ra in arthritis has
implicated pathophysiological and therapeutic aspects in multiple studies (Arend 2002).
Additionally, monosodium urate crystals in acute gout arthritis are potent activators of the
inflammasomes leading to the secretion of active IL-1β (Schroder and Tschopp 2010). The
synthetic human IL-1 receptor antagonist, anakinra is effective in the treatment of a murine
gout model, where complete resolution of arthritis is found in nine cases out of ten after
three days of treatment of acute gout (So et al. 2007). IL-1 inhibition has been considered to
be a promising treatment option for gout and larger clinical trials are underway with
rilonacept and canakinumab (Burns and Wortmann 2011).

An association between rheumatoid arthritis and an IL1B promoter variant in
Caucasians (rs16944) has been proposed while another IL1B coding variant in an Asian
population (rs1143634) was also linked (Harrison et al. 2008, Lee et al. 2009). A combined
analysis in three independent case-control cohorts and one longitudinal cohort was unable
to show a significant association with the IL-1 gene variation, but in one cohort a potential
novel IL1B variant (RA4) was associated with susceptibility of rheumatoid arthritis and the
incidence of radiographic erosions (Johnsen et al. 2008). A possible involvement of IL1A
and promoter IL1B SNPs are argued in studies of ankylosing spondylitis, although the
consistency and replication of the results are not supported by studies in different
populations (Maksymowych et al. 2006, Sims et al. 2008).

IL-1α and IL-1β both play an important role in bone metabolism (Lee et al.
2010). Osteoblast derived IL-1 is an important activator for the receptor activator of NF-κB
expression derived from osteoblasts while the bone-marrow-cell-derived IL-1 has specific
functions in the differentiation of premature osteoclasts. Some associations between IL-1
gene variation and osteoporosis phenotypes, mostly with regard IL1RN VNTR
polymorphism, have been described (Langdahl et al. 2000, Bajnok et al. 2000, Han et al.
2002, Chen et al. 2003, Kim et al. 2006). Additionally, IL1RN*2 has been reported to
associate with a decreased loss of bone mineral density while cross-sectional analyses
suggest an association with osteoporosis (Keen et al. 1998). Some forms of osteoarthritis
may associate with IL-1 gene variation, especially with IL1B variation (Moxley et al.
2010). Additionally, the selected candidate gene variants of IL1A and IL18 that are known
to interfere with connective tissue matrix synthesis and degradation have a suggestive association with intervertebral disc signal intensity (Videman et al. 2009).

2.2 GENES, DIABETES, INFLAMMATION AND CARDIOVASCULAR RISK

2.2.1 Candidate gene approach for studying complex genetic traits

Diseases caused by monogenic disorders are typically identified by using genetic linkage and positional cloning methods. However, identification of the genetic background of complex traits like metabolic dysregulation, type 2 diabetes (T2D), and cardiovascular disease suffers due to the lack of appropriate phenotype definitions and study populations (Tabor et al. 2002). The underlying mechanisms for the effects of the studied genes may vary and involve multiple biological pathways. Importantly, complex diseases are more likely to be caused by several, and even numerous, genes, each with a small overall contribution and relative risk.

The candidate-gene approach can be defined as the study of the influence on a complex trait by identifying genes that have a role in the aetiology of the disease and identifying variants in or near those genes. The associating variant might either lead to a change in the protein or its expression or be in linkage disequilibrium (LD) with the causal variants in a population. However, most susceptibility variants lie outside the coding regions of genes and are assumed to influence transcript regulation rather than gene function (McCarthy 2010). When two or more loci are not statistically independent they are said to be positively (or negatively) associated or in LD, that exists between loci because an insufficient number of generations have passed to allow recombination to randomize the haplotypes in the population (Shpak and Gavrilets 2005, Croucher 2008, Garnier-Ge´re´ and Chikhi 2008, Human Molecular Genetics 4th Edition 2011). Random drift can generate LD between markers and be responsible for differences at the population level. When a new mutation arises it will initially be in complete LD with one of the alleles of any neighbouring polymorphism. In a large, randomly mating population, LD is assumed to be mainly the result of physical linkage between close loci. LD with a longer distance can be generated by selection, where the fitness of an allele at one locus depends on that at another locus, and can lead to the preferential selection of certain haplotypes and therefore the maintenance of LD. Genetic drift due to bottlenecks or founder events may generate LD
with significant shifts in the distributions of haplotypes. Additionally, population admixture and stratification are sources for LD when the loci are holding different allelic frequencies between populations.

A single nucleotide polymorphism (SNP) is a fundamental genetic variation that is simple to study and contributes to phenotypic variation (Brookes 2007). Haplotypes have also been commonly used to localize a disease conferring gene or locus in association studies because haplotype analysis is suggested to be more powerful than linkage studies in localizing susceptibility loci for common diseases (Crawford and Nickerson 2005). Haplotypes are defined as a combination of alleles along the same chromosome. Several molecular methods are available to construct unambiguous haplotypes. A less expensive method of statistical inference for determining haplotypes from genotype data in large population-based surveys, including multiple generations, has recently improved substantially (Stephens and Donnelly 2003, Crawford and Nickerson 2005). The international HapMap Project was launched to provide a public resource for accelerating medical genetic research (International HapMap Consortium 2007). The Phase II HapMap further illustrated the fine-scale structure of linkage disequilibrium, recombination and natural selection by characterizing over 3.1 million human SNPs genotyped in 270 individuals from four geographically diverse populations while 25 to 35% of the common SNP variation was covered. The international HapMap Project has further characterized the genetic variation across different populations including rare SNPs, copy number variation, and selected sequencing, implicating that the lower-frequency variation is less shared across the populations and enables extended identification of genes associating with diseases and traits (International HapMap 3 Consortium 2010).

Biological plausibility of association, its consistency with existing knowledge about biology, and the strength of association, have been considered as the guidelines for candidate gene studies in assessing the role of the associations with complex traits (Tabor et al. 2002). Dose–response relationships of the associations across studies and different populations are important considerations while consistent replications in different populations markedly increase the evidence for causality.
2.2.2 Genome-wide association studies

Genome-wide association studies (GWAS) have used several hundred thousands to 2.5 million SNPs in more than 100,000 individuals for studies of complex diseases and traits (Manolio 2010). An estimated 10 million common SNPs with minor allele frequency of at least 5% are transmitted across generations allowing a few tagging SNPs to capture the majority of genetic variation within each haplotype block. A GWA scanning can be used in various study designs including case-control studies, cohort studies, and clinical trials. The large number of genetic association tests necessitates correction for multiple testing (Hirschhorn and Daly 2005). Other strategies to avoid false positive findings include; replication in multiple populations to deal with bias due to population stratification and bias due to technical artefacts.

In GWAS it is presently assumed that common diseases are attributable in part to allelic variants that are relatively common with a frequency more than 1 to 5% (Manolio 2009). Individual SNPs, that are situated most often in noncoding regions alone or in combination, confer relatively small increments in risk and explain only a small proportion of heritability. Much of the discussion on missing heritability in GWAS has been focused on the possible contribution of variants with low minor allele frequency (MAF), usually less than 1%. The primary technology for detecting and studying these rare SNPs may require ‘next-generation’ deep-resequencing methods. Other caveats in defining the heritability of complex diseases include the small number of studies using structural genetic variants and non-European populations.

2.2.3 Defining inflammation

Inflammation is characterized as vascular and cellular responses to noxious stimuli and conditions related to infections or tissue injury. The major task for innate immunity is to recognize and deal with foreign microbial antigens and intrinsic antigens that result from cellular damage or death. Antigen specific responses to pathogens are mediated by cellular and humoral immune responses and adaptive immunity is considered to be a major contributor to immune memory and immunologic tolerance that is pivotal in the recognition of non-pathogenic structures and self-antigens. The immune system is composed of mechanisms for pattern recognition, antimicrobial peptides, immune cells, the complement
Inflammatory mediators are classified into seven groups according to their biochemical properties: vasoactive amines, vasoactive peptides, fragments of complement components, lipid mediators, cytokines, chemokines and proteolytic enzymes (Robbins and Cotran Pathologic Basis of disease 8th Edition 2010).

Inflammation is largely initiated by signals from pattern recognition receptors (PRRs) that respond to evolutionarily conserved molecular signatures of microbe-like figures, known as pathogen-associated molecular patterns (PAMPs). However, the events leading to chronic inflammation and autoimmune diseases are only partly understood and the causes and mechanisms of systemic chronic inflammation which occur in a wide variety of diseases, including metabolic dysregulation, type 2 diabetes, and cardiovascular diseases, are known to an even lesser extent (Medzhitov 2008). Additionally, the homeostatic control during a stress response or healing phase, may be mediated primarily by tissue-resident and recruited macrophages, while cell death or injury may cause a disrupted plasma membrane resulting in the release of certain cellular constituents that activate the inflammasome cascade.

2.2.4 Glucose abnormalities, cardiovascular diseases and prolonged inflammation

2.2.4.1 Subclinical inflammation and glucose homeostasis

The failure of beta cells to compensate for insulin resistance is commonly regarded as the leading causative factor in the development of type 2 diabetes (T2D). Furthermore, accumulating evidence shows that inflammation is also a key feature of obesity and T2D (Wellen and Hotamisligil 2005, Kolb and Mandrup-Poulsen 2005). Inflammatory activity in white adipose tissue is up-regulated as shown by the expression of inflammatory genes studied in multiple tissues (Xu et al. 2003). Additionally, obesity is known to associate with macrophage infiltration and overproduction of tumour necrosis factor alpha TNFα in adipose tissue (Weisberg et al. 2003, Hotamisligil 2006).

Mechanisms underlying the insulin resistance seen in metabolic syndrome are related to the overabundance of circulating fatty acids, glucose and triglycerides (Eckel et al. 2005, Kolb and Mandrup-Poulsen 2005). Additionally, altered mitochondrial oxidative phosphorylation is related to lipid accumulation with the effects on the serine
phosphorylation of IRS-1 associated with protein kinase C (PKC) activation in liver and muscle tissues. Moreover, PKC and NF-κB are assumed to play a specific role in the development of insulin resistance after lipid infusions or high-fat diet following IL-1β/TNFα expression and diacylglycerol accumulation in the liver (Boden et al. 2005, Samuel et al. 2010). Hepatic steatosis and subacute inflammation with increased NF-κB activity follows after a high-fat diet (Cai et al. 2005). Interactions with immune mechanisms are reported to exert counter regulation with neuroendocrine and sympathetic control of the hypothalamic-pituitary-adrenal axis (Pickup 2004, Kershaw and Flier 2004).

An increased number of immune cells, specifically macrophages, are found in human T2D tissue specimen samples (Ehses et al. 2007, Richardson et al. 2009). Cultured islets exposed to T2D milieu or isolated from high-fat fed mice show an increased production of cytokines and chemokines related to macrophage recruitment. Furthermore, pancreatic islet cells confer remarkable capacity to adapt to conditions of increased insulin demands in prediabetes leading to beta cell deficiency and apoptosis in overt diabetes (Donath et al. 2003, Donath et al. 2005). Elevated glucose and free fatty acid (FFA) levels are considered major contributing factors for beta cell apoptosis resulting from a high-caloric diet induced metabolic stress (Kolb and Mandrup-Poulsen 2005).

Virtually all systemic indicators of subclinical inflammation are reported to be elevated in T2D while most studies propose that the degree of immune activation is far below that seen in acute infections (Pickup 2004, Kolb and Mandrup-Poulsen 2005). The indicators for subclinical inflammation in T2D include increases in acute-phase proteins, cytokines and mediators that are associated with markers of endothelial activation (Duncan et al. 1999, Schmidt et al. 1999). Proinflammatory activation and modest increases of circulating inflammatory mediators have been reported many years before the diagnosis of T2D (Kolb and Mandrup-Poulsen 2005). An increasing number of metabolic syndrome components are associated with increased levels of C-reactive protein (CRP) and interleukin-6 (IL-6). Increased levels of the cytokine-like inflammatory marker, monocyte chemo-attractant protein-1 (MCP-1) were associated with the development of T2D after adjustment for multiple covariates including CRP and IL-6 levels (Herder et al. 2006).

Increased glucose levels are reported to be capable of inducing proinflammatory cytokine and chemokine gene expression in monocytic cells (Shanmugam
The evidence for hyperglycaemia being capable of inducing inflammation in T2D is further supported by analyses where IL-6 and CRP correlate with blood glucose or glycated haemoglobin levels (Pickup 2004). Oxidative stress has been shown to be enhanced in hyperglycaemia together with a metabolically decompensated state in diabetes (Libby 2002, Goldberg 2009). In vitro and in vivo studies have provided evidence to suggest that, in various tissues, hyperglycemia and possibly elevated FFA levels (both alone and in combination) result in increased oxidative stress (Evans et al. 2003). Reactive oxygen species may not only directly cause cell damage, but indirect mechanisms may also be involved, activating a variety of stress-sensitive intracellular signalling pathways including; NF-κB, PKC, advanced glycation end product (AGE) or receptor for advanced glycosylated endproducts (RAGE), mitogen activated protein kinases (MAPK) and extracellular receptor kinases (ERK).

Sex differences in inflammatory markers and the development of diabetes have been suggested. CRP is a considerably stronger predictor for diabetes in women, suggesting an interaction between sex and inflammatory response (Thorand et al. 2007). Other studies have not uniformly supported this observation however. The proposed interaction of inflammatory markers and gender may be related to a stronger correlation between adiposity and inflammation in women than in men (Thorand et al. 2006). Folsom et al. (2005) studied endogenous sex hormones and their carrier proteins in postmenopausal women and found increased CRP levels associating with increased estrone, increased androstendione and decreased sex hormone binding globulin (SHBG) levels (Folsom et al. 2005). Meta-analysis of sex hormones affecting T2D risk shows that increased testosterone levels are associated with elevated risk in women and decreased risk in men; increased SHBG levels were inversely associated with T2D risk (Ding et al. 2006). Elevated endogenous estradiol levels are associated with the presence of diabetes in men and postmenopausal women. A prospective study of incident diabetes in postmenopausal women also found an association with increased levels of bioavailable testosterone, estradiol and decreased SHBG levels (Kalyani et al. 2009). These associations were explained to varying degrees by adiposity and insulin resistance and were also previously associated with T2D and impaired fasting glucose (IFG) in a cross-sectional analysis of the same cohort (Golden et al. 2007). The literature regarding estrogen and glucose
homeostasis is mixed, however, and longitudinal studies with repeated measurements are lacking. The association of increased CRP levels with oral hormone replacement therapy in postmenopausal women has been reviewed (Lakoski and Herrington 2005). Nonetheless, the mechanism by which oral conjugated estrogen replacement therapy alone or with progestins can increase CRP levels remains unresolved, although the effects were not mediated by IL-6 or markers of endothelial dysfunction (Hu et al. 2006).

2.2.4.2 Therapeutic effects on inflammation

Common treatment strategies for T2D display a decreasing trend for inflammatory parameters following dietary interventions, weight loss, physical exercise and pharmacological antidiabetic treatments including sulphonylureas, metformin, glitazones or insulin (Kolb and Mandrup-Poulsen 2005). Furthermore, anti-inflammatory agents such as high dose salicylates have long been known to lower glycosuria in diabetic patients (Pickup 2004). Inhibition of nuclear factor-κB (NF-κB) and its upstream activator IKKβ have been reported as the underlying mechanism for the blood glucose decreasing effects of salicylates. Indeed, T2D patients treated with a very high dose of acetyl salicylic acid show improvements in fasting and postprandial hyperglycaemia (Yuan et al. 2001, Hundal et al. 2002). In addition to the inhibition of cholesterol synthesis, statins have been proposed to affect intracellular signalling cascades, which may have an impact on cardiovascular risk reduction (McFarlane et al. 2002). Despite the possible anti-inflammatory role of statins they were found to cause a slightly increased risk of diabetes in a recent comprehensive meta-analysis (Sattar et al. 2010). Pharmacologic interruption of the renin–angiotensin system (RAS) using angiotensin-converting-enzyme inhibitors or angiotensin receptor antagonists may interfere with insulin signalling and may be related to the unfavourable effects of angiotensin II (Prasad and Quyyumi 2004). Blocking of RAS has been found beneficial in the prevention of glucose abnormalities, hypertension, heart failure and coronary artery disease in multiple studies (Abuissa et al. 2005, Gillespie et al. 2005).
2.2.4.3 Inflammation in diabetes, metabolic syndrome and cardiovascular diseases

Vascular injury and endothelial dysfunction is regarded to be an early event in the development of atherosclerosis by promoting inflammation with increased endothelial leukocyte recruitment, adhesion and permeability (Ross 1999). Nitric oxide derived from endothelium is known to interact with vascular cell adhesion molecule (VCAM) expression associated with NF-κB activation (Libby 2002). Atherosclerotic lesions harbour an accumulation of immune cells that interact with both matrix components and the immune system. Similarly, modified LDL and other lipoproteins are retained to the lesion where they also interact with matrix components (Lusis 2000). Many risk factors have been reported to stimulate smooth muscle cells, foam cells and formation of atherosclerotic plaques in the vascular wall milieu. Elevated blood pressure and alterations in arterial flow also affect the level and localization of inflammation, further augmenting the progression of atherosclerosis (Ross 1999). Furthermore, the progression of atherosclerosis is accompanied by microvessel formation and thrombosis in the subendothelial vascular plaques (Libby 2002).

Indicators for chronic subclinical inflammation are regarded to be independent risk factors for cardiovascular disease. CRP levels in particular, are proposed to provide more accurate prognostic information as compared to the traditional cardiovascular risk factors or LDL cholesterol levels alone (Willerson and Ridker 2004). The interrelationships seen between cardiovascular events, metabolic syndrome and CRP levels in women suggest that increased inflammation in metabolic syndrome could amplify risk for forthcoming cardiovascular events. Endothelial dysfunction and chronic low-grade inflammation are related to cardiovascular events in T2D (Goldberg 2009). Impaired endothelium-dependent vasodilatation is regarded to be an early phenomenon of vascular injury in metabolic dysregulation or diabetes. Endothelial dysfunction is related to the presence of hyperglycaemia, insulin resistance, increased free fatty acids or dyslipidemia further promoting the progression of atherosclerosis with activation of intracellular cascades, increased oxidative stress and activation of the receptors for advanced glycation end-products (Beckman et al. 2002). The mechanisms responsible for the increased cardiovascular disease risk that accompanies T2D were also studied in an extended follow-up of a population based cohort (de Jager et al. 2006). A substantial fraction of the
cardiovascular risk burden was explained by the increased levels of circulating markers that are related to endothelial dysfunction and inflammation, especially in cases of T2D.

The presence of T2D is known to increase the risk for cardiovascular morbidity and mortality by 2- to 4-fold (Beckmann et al. 2002). The incidence in fatal coronary artery disease is accordingly found to be increased in a large meta-analysis of T2D individuals with 50 % higher relative risk in women as compared to men (Huxley et al. 2006). The same study reported that, increased cardiovascular risk profile and decreased number of standard treatments are found in women as compared to men. A recent comprehensive meta-analysis of almost 700 000 people reported that after accounting for other risk factors, diabetes more than doubled the risk for coronary heart disease and stroke (Emerging Risk Factors Collaboration (A) 2010). Additionally, the presence of IFG independently increased the risk for coronary heart disease by 15 % in women and 7 % in men. Pre-diabetes and the risk for cardiovascular disease have been reviewed in systematic meta-analysis showing that both IFG and impaired glucose tolerance (IGT) modestly increase the risk estimates without significant sex differences (Ford et al. 2010). In addition to vascular disease, diabetes is also associated with premature mortality of several cancers, infectious diseases, injuries, suicide and various degenerative disorders that are independent of several major risk factors (Emerging Risk Factors Collaboration (B) 2010). Overall, worsening of the short- and long-term risk estimates for cardiovascular events has been observed in the presence of diabetes while the localization of vascular events was affected especially when diabetes is present at a young age (Beckmann et al. 2002).

Metabolic syndrome is known as a significant risk factor for forthcoming diabetes while predicting to a lesser extent incident cardiovascular events and mortality (Ford 2005, Gami et al. 2007, Pajunen et al. 2010). The strong capability of metabolic syndrome to predict diabetes is, however, not found to improve prediction beyond its components while impaired fasting glucose and increased waist circumference were among the strongest predictors for T2D (Ford et al. 2008). Notwithstanding the recent doubts for the usefulness of MetS, it can still be considered as a useful tool to outline metabolic dysregulation and the risk for development of T2D (Reaven 2011). Additionally, abdominal obesity has an impact on diabetes frequency across Europe despite regional differences in CVD rates and risk factors (Fox et al. 2009). Metabolic risk profiles or dysregulation in
childhood are potential contributors for risk prediction of adult T2D and a substantial increase in the prevalence of metabolic syndrome among healthy young adults in the recent three decades has been reported (Mattsson et al. 2006). Despite the instability in the diagnosis of youth metabolic syndrome, the dichotomous definitions in childhood predict important disease outcomes of T2D in early to middle adulthood (Magnussen et al. 2010). Furthermore, an important finding was that high body mass index in childhood predicts T2D as well as or better than the categorical metabolic syndrome definitions. The definition for metabolic syndrome using International Diabetes Federation (IDF) criteria has taken into account the global variation of waist circumference, cardiovascular disease burden and risk for the development of diabetes including national and regional cut-off points (Eckel et al. 2010). Insulin resistance as a major feature in metabolic dysregulation has been closely linked to central obesity further guiding the development of international standardized definitions for metabolic syndrome.

Glycated haemoglobin (HbA1c) has been validated as a new risk factor for incident diabetes independent of fasting glucose levels as reported in a large study of middle-aged individuals (Selvin et al. 2010). A HbA1c level above 6.0 % is also described as a marker for increased cardiovascular morbidity and mortality. The observed J-shaped relation in cardiovascular risk prediction may have resulted from unknown factors interfering with HbA1c levels in the state of low-normal glycaemia. Fasting plasma glucose and 2 hour post load glucose measurements were found to have higher short-term variability when compared to the variability exerted from HbA1c measurements (Selvin et al. 2007). The latest diagnosis and classification of diabetes mellitus by the American Diabetes Association has recommended glycated haemoglobin (A1C) values between 5.7 to 6.4 % as indicators of increased risk for diabetes and values more than or equal to 6.5 % as a criterion for diabetes (American Diabetes Association 2010).

2.2.4.4 Systemic inflammation and subacute thyroiditis
A systemic inflammatory response with fever, elevated serum CRP and increased erythrocyte sedimentation rate (ESR) has been commonly found in subacute (De Quervain) thyroiditis (SAT) (Singer 1991). This painful and self-limiting granulomatous disorder of the thyroid gland with thyrotoxicosis is followed by transient hypothyreosis and sometimes
mimicks other causes of fever of unknown origin. Permanent hypothyreosis is reported in 5 to 10% of cases and transient expression of thyroid antibodies has previously been reported with SAT (Volpe 1993). Non-steroidal anti-inflammatory drugs are typically used to treat SAT and corticosteroids have been reserved for more severe cases. Beta-adrenergic blocking drugs can be given to patients until hyperthyroidism resolves. SAT is considered to be of viral origin; direct evidence of the presence of viruses or their components in the thyroid gland has been reported for retroviruses and mumps (Desailloud and Hober 2009). It is proposed that thyroid tissue could respond with thyroiditis after invasion by a variety of different viruses without any single agent likely to be causative.

During the acute phase of SAT the symptoms of mild hyperthyroidism may be followed by transient hypothyroidism in the late phase, possibly lasting from several weeks to a few months. SAT is accompanied by subnormal serum free thyroxine or free tri-iodothyronine levels, and elevated thyrotropin (TSH) levels (Volpe 1993). In the early phase of the disease, the serum free thyroxine levels are proportionately elevated, leukocyte count is generally normal and ESR is markedly elevated. Thyroid ultrasound examinations and fine-needle aspiration biopsies are used to support the diagnosis. Distress, malaise, discomfort, and pain may last for several weeks while a physical examination typically shows a febrile patient with an exquisitely tender, firm and ill-defined nodular thyroid lesion. Thyroid follicles are infiltrated with predominantly mononuclear cells and show disruption of the epithelium and a loss of colloid leaking into the interstitial tissue followed by inflammatory reaction (Singer 1991, Volpe 1993). The central core of colloid is surrounded by multinucleate giant cells with progression to granulomatous formation. The destruction of epithelium and loss of follicular integrity is known to lead to the release of thyroid hormones to the circulation.

Clinical features and outcomes of SAT have been reviewed extensively in a population based follow-up study (Fatourechi et al. 2003). The overall age and sex-adjusted incidence of SAT was reported; 4.9 cases per 100,000 yearly from 1960 through 1997. Among SAT cases 26% received corticosteroid therapy and nonsteroidal anti-inflammatory drugs alone were given in 41% of the cases. A decreasing trend for the incidence of SAT was found during the follow-up in women. Late hypothyroidism after a 10-year follow-up was found in 4% of cases, and after 28 years the incidence was 9.5%.
associated with corticosteroid treatment. Autoimmune diseases associated with SAT have been found in 5% of cases, including involvement of gastrointestinal, rheumatic and skin disorders. There was a trend towards an increasing number of cases in fall and spring, but the statistical significance was not shown in a community-based follow-up. SAT is considered to be as a rare form of hyperthyroidism, assumed to develop in the summer months, arising much more frequently in women, and it has been strongly associated with the human leucocyte antigen (HLA) B35 genotype together with reports of familial occurrence (Cooper 2003, Kramer et al. 2004). Radioactive iodine uptake by the thyroid is usually decreased in SAT. Milder forms of biochemical hyperthyroidism have been identified in SAT, as compared to Graves’ disease when assessed with the ratio of thyroxine to tri-iodothyronine (Cooper 2003). The prevalence of thyroid peroxidase (TPO) and thyroglobulin (TG) antibodies is increased in cases of SAT with spontaneous hypothyroidism, exceeding 95%, while the presence of thyroid antibodies is evident in approximately 70% of permanent hypothyroidism cases (Carle et al. 2006). Despite the overall increase in the incidence of hypothyroidism in recent years, the incidence of SAT has not changed and the unadjusted incidence in women is 1.0 case per 100,000 per year, and in men 0.2, respectively (Carle (B) et al. 2006). CRP levels are significantly higher in subjects with the diagnosis of SAT when compared to other forms of thyroiditis (Pearce et al. 2003).

2.2.5 Genetics of type 2 diabetes

The genetic background of T2D is supported by the occurrence of familial clustering of insulin sensitivity and secretion, the higher concordance rate of T2D in monozygotic versus dizygotic twins and the high prevalence of T2D in certain ethnic groups (Doria et al. 2008, Grarup et al. 2010). Most of the monogenic forms of diabetes with strong effect sizes are rare and the rare syndromes currently account for less than 5% of diabetes cases; while the heterogeneous inheritance pattern of T2D was postulated to evolve from a complex interplay of many different pathways under the combined control of environmental and genetic factors. However, the role of the environmental and health behavioural factors in the development of monogenic forms of diabetes is fairly small while the age of the onset of diabetes is an important clue for specific genetic syndromes (Laakso 2011). The
diagnosis of Maturity Onset Diabetes of the Young type 1 or 3 (MODY1 or MODY3) facilitates clinical follow-up and treatment for the prevention of diabetic complications. Additionally mutations in a number of genes warrant a specific pharmacological treatment, including; potassium inwardly-rectifying channel, subfamily J, member 11 (KCNJ11) and ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8). Currently, the clinical feasibility of genetic testing for diabetes is limited to only monogenic forms.

The candidate variants of SNPs for predicting T2D derived from the Human Genome Project have been deposited in public databases (Doria et al. 2008, Florez 2008). Association analyses have been regarded more powerful than linkage analysis, although the signal can be detected only if one examines the causal variant itself or a nearby marker with a strong LD (McCarthy 2010). Researchers have more recently directed their attention to specific candidate variants or genes of interest that emerged from genome-wide studies. The genetic variation of Calpain-10 (CAPN10) is associated and linked with T2D suggesting that variation in CAPN10 affects the risk for T2D in Europeans (Horikawa et al. 2000, Tsuchiya et al. 2006). The name Calpain is derived from a reflection on families of calcium dependency and sequence homology with a family of cysteine proteases. Calpain-10 has is suggested to exert multiple actions in pancreatic beta cells and also to contribute to insulin resistance through peripheral mechanisms (Turner et al. 2005). A modest linkage peak for T2D in chromosome 20 is replicated in multiple populations and a candidate region has subsequently been comprehensively genotyped and analyzed. Another locus in chromosome 1 is undergoing a detailed fine-mapping and association analysis with T2D related traits (Florez 2008). A recent genome-wide linkage study in African-American families describes the strongest signal for T2D in chromosome 2 (LOD 4.53), a broad peak extending from 41 to 121 Mb including the candidate gene families IL-1 and IL-1R (Elbein et al. 2009). The strongest effect on T2D risk yet described, results from identification of linkage signals in the gene encoding the transcription factor 7-like 2 (TCF7L2) in chromosome 10 and this robust association has been replicated in almost every population examined (Grant et al. 2006, Florez 2008). TCF7L2 is established as a transcription factor that plays an important role in the canonical Wnt (wingless-related gene, a list of genes encoding Wnt signalling proteins) signalling pathway that affects a wide range of important cellular processes. An important interplay is proposed between incretin hormone glucagon-
like peptide-1 and TCF7L2 in the regulation of pancreatic islet functions (Hansson et al. 2010). The different strategies for identifying T2D genes are listed in Table 4.

Multiple GWAS in populations of European ancestry have identified loci where common variant SNPs reproducibly influence the risk of T2D including relevant quantitative and intermediary traits. The large scale meta-analysis study of over 10 000 individuals and approximately 2.2 million directly genotyped or imputed SNPs was followed by an independent replication sample of up to 54 000 individuals confirming previously known and identifying additional susceptibility loci for T2D (Zeggini et al. 2008). The meta-analysis of 21 performed GWAS in individuals without diabetes identified multiple loci associated with glucose homeostasis traits and confirmed the earlier observations including associations with T2D (Dupuis et al. 2010). The majority of identified variants were related to beta cell function while only two variants were associated with insulin sensitivity including a strong biological candidate, the insulin receptor substrate -1 (IRS-1) locus associated with a homeostasis model assessment for insulin resistance (HOMA-IR). The likely biological candidate genes in these loci were found to influence signal transduction, cell proliferation and development, glucose-sensing and circadian regulation. However, one should also consider the possibility that not all loci associated with fasting glucose within the “physiological” range are also associated with “pathological” fasting glucose levels and T2D risk. Therefore the mechanism by which glucose is raised is an important consideration.

The coding variants in peroxisome proliferator-activated receptor gamma (PPARG) and KCNJ11 have modest effects on the risk of type 2 diabetes while the genes are known as functional targets for classes of therapeutic agents widely used in diabetes management (McCarthy 2010). Currently, a total of approximately 40 confirmed T2D associated loci have been identified. These loci include variants in or near Wolfram syndrome 1 (wolframin) gene (WFS) and the hepatocyte nuclear factors HNF1 homeobox A (HNF1A) and HNF1B homeobox B (HNF1B) that also harbour rare mutations responsible for monogenic forms of diabetes. Furthermore, common variation in insulin receptor substrate 1 (IRS1) has been found with a primary effect on insulin action rather than secretion. The fat mass and obesity associated (FTO) gene variation was proposed to have impact on T2D by an intermediate effect on body-mass index, adiposity and insulin
resistance (McCarthy 2010). Other signals with effects on body mass index reinforce the likelihood that obesity is a disorder of hypothalamic function. In GWAS, quantitative traits have been used for the identification of genetic factors in overt disease, and simple surrogate measures for insulin resistance have been considered appropriate for large-scale clinical and epidemiological studies with cross-sectional design (Buchanan et al. 2010). The genetic loci identified to associate with glucose homeostasis traits show considerable heterogeneity and therefore the use of different measures to treat insulin sensitivity requires cautious consideration: this highlights the fact that their underlying genetic physiology requires further study (Ingelsson et al. 2010).

Individuals with elevated glucose levels and IFG or IGT are at increased risk for diabetes; the glycated haemoglobin values have also been used for diabetes risk prediction (American Diabetes Association 2010). Overweight individuals, with body-mass index equal to or more than 25 kg/m$^2$, are considered to have increased risk for T2D, especially if accompanied by low physical activity, family history of diabetes, hypertension, low HDL cholesterol, elevated triglycerides and obesity. Additionally, an analysis of middle-aged subjects showed that clinical information together with lipid and fasting glucose measurements were able to characterize groups with varying degrees of diabetes risk (Schmidt et al. 2005). Simple models effectively predict the development of T2D, with no evident improvements in the risk prediction even after extended modelling (Wilson et al. 2007). The loci suspected for the familial basis of diabetes have also been combined into a genetic risk score to improve the detection of persons at risk for diabetes (Weedon et al. 2006). The addition of known common genotypes produced only a minimal improvement in the assessment of risk estimation for T2D when added to the phenotype based risk models (Lyssenko et al. 2008, Meigs et al. 2008, Talmud et al. 2010). It should be noted that prospective studies on the risk of incident T2D are almost totally lacking at the moment. Nevertheless, risk-allele discovery has led to an improved understanding of the biologic basis of disease (McCarthy 2010). Therefore, information from genetic studies could be used to identify new targets for pharmaceutical intervention that have validated effects on physiological characteristics. Additionally, novel findings could provide information about new targets and promote the characterization of high-risk groups to
enable more efficient clinical trials of interventions that prevent metabolic dysregulation, the development of T2D or their complications.

The genetic background for T2D may be more polygenic and heterogeneous than previously believed. The frequencies of the identified variants are relatively high at the population level, but these loci explain only a small proportion of the familial clustering on T2D (Doria et al. 2008). Additionally, other proposed hypotheses for the genetic background of T2D include gene-gene interactions, epigenetic effects and effects of rare variants with a minor allele frequency of less than 1%. It has been suggested that new elements for genetic susceptibility are expected to evolve in the future, because of the rather low proportion of variance explained by GWAS findings. The variants with a relatively low minor allele frequency may have an impact on certain subsets of the population and selected sequencing studies of the quantitative trait of interest may be needed to uncover the missing heritabilty of T2D (McCarthy 2010). The specific physiologic defects could then be investigated to supplement to the association studies at the population level.
**Studies of monogenic forms of type 2 diabetes:**
- Maturity Onset Diabetes of the Young (MODY)
- Rare forms of severe insulin resistance
- Neonatal diabetes
- Mitochondrial syndromes of diabetes

**Candidate gene approach:**

*Functional*
- Genes involved in insulin action and insulin secretion
- Genes associated with diseases associated with diabetes (obesity, genetic syndromes, etc)
- Genes identified in animal models of diabetes

*Positional*
- Genes in linked intervals identified through family studies
  - Useful for monogenic forms of diabetes (e.g., MODY)
  - Challenging for common, multifactorial Type 2 diabetes

**Finding genes with altered levels of expression:**
- Subtraction cloning and differential display
- Microarray analysis (gene expression arrays)

**Genome-wide association studies:**
- Microarray-based typing of SNPs spanning the genome
  - Large populations of diabetic cases and non-diabetic controls
  - Populations with intermediate phenotypes – obesity, insulin resistance, polycystic ovarian disease
3. AIMS OF THE STUDY

The general aim of this investigation was to analyze IL-1 gene variation and the IL-1 receptor antagonist phenotype in immunological responses, metabolic dysregulation and type-2 diabetes. The specific aims for this study were:

1. To investigate the associations of IL-1 gene variation and IL-1Ra in the development of thyroid antibodies in consecutive cases of subacute thyroiditis and to study the role of the gene variants in the development of permanent hypothyroidism after the average follow-up of 2.4 years.

2. To investigate the associations of common genetic variants in the IL-1 gene family with plasma glucose and insulin concentrations, indices of insulin resistance and beta cell function, and prevalent diabetes in a representative population sample. The findings were tested for replication in a cohort of European myocardial infarction survivors and the consistency was checked by comparing the results with the “gray area” findings of the earlier GWAS studies.

3. To investigate genetic factors and non-genetic individual characteristics determining the systemic IL-1Ra phenotypes in a cross-sectional setting in three independent study populations that possess data on common IL-1 gene variation as well as on the constitutive factors and metabolic traits.

4. To investigate (a) whether the IL-1 gene variants are predictors for clinically incident diabetes in a prospective setting; and (b) whether the IL-1Ra levels differ between non-diabetic individuals with and without metabolic syndrome and whether they predict the progression to overt diabetes during the follow-up of persons with metabolic syndrome.
4. MATERIALS AND METHODS

4.1 STUDY SUBJECTS AND SETTING

4.1.1 Subacute thyroiditis patients and controls

A group of 54 consecutive patients with subacute thyroiditis were prospectively studied. The diagnostic criteria for subacute thyroiditis (SAT) were; painful thyroid palpation, fever and neck pain together with laboratory tests indicating acute systemic inflammation or abnormal thyrotropin and free thyroxin levels. Corticosteroid therapy was commenced immediately to relief pain and inflammatory symptoms. Diagnosis was further supported by thyroid ultrasonographic examination and fine-needle aspiration biopsies. Baseline data of 54 patients with SAT are shown in Table 5. Baseline blood samples were taken at the first visit. Control examinations with laboratory tests were carried out on 48 subjects after 2.42 years (mean) ranging from 0.67 to 3.83 years. Four hundred healthy blood donors of the same ethnic origin were used as controls at baseline examination. The study was approved by local ethics committees and written consent was given by all participants.
Table 5. Clinical and laboratory data of 54 subjects with the diagnosis of subacute thyroiditis.

<table>
<thead>
<tr>
<th></th>
<th>Result</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, [mean (range)]</td>
<td></td>
<td>49 (19–79)</td>
</tr>
<tr>
<td>Sex</td>
<td>Female/Male</td>
<td>35/19</td>
</tr>
<tr>
<td>Thyrotropin (mU/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.05</td>
<td></td>
<td>31 (57.4 %)</td>
</tr>
<tr>
<td>0.05 – 0.4</td>
<td></td>
<td>9 (16.7 %)</td>
</tr>
<tr>
<td>0.4 – 6.0</td>
<td></td>
<td>8 (14.8 %)</td>
</tr>
<tr>
<td>&gt; 6.0</td>
<td></td>
<td>6 (11.1 %)</td>
</tr>
<tr>
<td>Free thyroxin (pmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 20.0</td>
<td></td>
<td>37 (68.5 %)</td>
</tr>
<tr>
<td>9.0 – 20.0</td>
<td></td>
<td>16 (29.6 %)</td>
</tr>
<tr>
<td>&lt; 9.0</td>
<td></td>
<td>1 (1.9 %)</td>
</tr>
<tr>
<td>ESR (mm/h) (mean ± SEM)</td>
<td>73.4 ± 4.4</td>
<td>&gt; 20 mm/h:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51 (94.4 %)</td>
</tr>
<tr>
<td>C-reactive protein (mg/l) (mean ± SEM)</td>
<td>56.6 ± 5.3</td>
<td>&gt; 10 mg/l:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>43 (79.6 %)</td>
</tr>
<tr>
<td>Cytology of fine-needle aspiration sample</td>
<td>Consistent with SAT</td>
<td>33 (61.1 %)</td>
</tr>
<tr>
<td></td>
<td>Suggestive of SAT</td>
<td>10 (18.5 %)</td>
</tr>
<tr>
<td></td>
<td>Inadequate for diagnosis</td>
<td>11 (20.4 %)</td>
</tr>
<tr>
<td>Ultrasonography of the thyroid</td>
<td>Diagnostic findings</td>
<td>52 (96.3 %)</td>
</tr>
<tr>
<td>Therapy</td>
<td>Corticosteroids</td>
<td>43 (79.6 %)</td>
</tr>
</tbody>
</table>

ESR - erythrocyte sedimentation rate, SEM - standard error of mean.
Normal range for thyrotropin: 0.4 - 6.0 mU/l
Normal range for free thyroxin: 9.0 - 20.0 pmol/l
4.1.2 Health 2000 population

The Health 2000 survey was based on a national representative population sample of 8,028 men and women aged 30 or over (Aromaa and Koskinen 2004). The participation rate was 84.3%. Participants were recruited using a two-stage stratified cluster sampling from the National Population Information System to represent the total population of Finland aged 30 and above. The sampling frame comprised adults living in mainland Finland. This frame was regionally stratified according to the five university hospital regions, each containing roughly one million inhabitants. From each university hospital region 16 health care districts were sampled as clusters. Persons aged 80 years and over were oversampled by doubling the sampling fraction. Altogether, 6,771 persons participated in laboratory investigations and gave an informed consent. Anthropometric measurements included height, weight, and waist circumference. After a five minute rest, blood pressure was measured twice from the right arm whilst the person was sitting. Laboratory characteristics of glucose homeostasis in the Health 2000 participants are shown in Table 6. Baseline clinical data is shown in Table 7 including the 2 hour oral glucose tolerance test (OGTT) on a subsample of 625 men (mean age 56.4, range 45 – 74) and 765 women (mean age 56.9, range 45 – 74) living within a reasonable distance from a university hospital. The original cardiovascular and diabetes oriented subsample consisted of 1,826 individuals and finally included 1,390 individuals with OGTT. The Health 2000 Study has been approved by the appropriate ethics committees and the participants gave a written, informed consent. Follow-up of the Health 2000 study included 5,511 subjects aged 30 to 74 years. Their baseline characteristics are shown by gender in Table 8. Persons with prevalent diabetes or self-reported impaired glucose tolerance at baseline were excluded from the longitudinal analyses.
Table 6. Laboratory characteristics of the Health 2000 study population (n = 6 771) and the subsample with oral glucose tolerance tests (OGTT).

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic (n = 6 176)</th>
<th>mean (IQR)</th>
<th>Non-medicated diabetic (n=301)</th>
<th>mean (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fP-insulin, pmol/l</td>
<td>46.8 (34.7–69.4)</td>
<td>fP-insulin, pmol/l 95.0 (69.4–138.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fP-glucose, mmol/l</td>
<td>5.31 (5.0–5.6)</td>
<td>fP-glucose, mmol/l 7.31 (6.3–8.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>11.1 (6.9–16.7)</td>
<td>HOMA-IR 30.5 (19.4–47.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-βcell</td>
<td>523.6 (52.6–365.3)</td>
<td>HOMA-βcell 536.8 (387.5–767.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.20 (5.0–5.4)</td>
<td>HbA1c, % 7.29 (6.4–8.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OGTT subsample laboratory

<table>
<thead>
<tr>
<th></th>
<th>mean (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-insulin 0 min, pmol/l</td>
<td>57.8 (39.6–77.8)</td>
</tr>
<tr>
<td>P-insulin 30 min, pmol/l</td>
<td>297.2 (200.7–430.6)</td>
</tr>
<tr>
<td>P-insulin 120 min, pmol/l</td>
<td>272.2 (170.1–434.1)</td>
</tr>
<tr>
<td>P-glucose 0 min, mmol/l</td>
<td>5.77 (5.3–6.1)</td>
</tr>
<tr>
<td>P-glucose 30 min, mmol/l</td>
<td>8.68 (7.7–9.8)</td>
</tr>
<tr>
<td>P-glucose 120 min, mmol/l</td>
<td>6.58 (5.1–8.0)</td>
</tr>
</tbody>
</table>

1 Geometric mean with interquartile range (IQR), 2 Hypoglycemic medication in 22 (1.6%)

4.1.3 European myocardial infarction survivors

A multicenter study “Air Pollution and Inflammatory Response in Myocardial Infarction Survivors: Gene-Environment Interaction in a High Risk Group” (AIRGENE) included 972 individuals from six European cities, Athens, Augsburg, Barcelona, Helsinki, Rome and Stockholm, aged 35 to 80 years (mean 62) with a female to male ratio of 0.27. Potential participants for the study were identified from population-based registries of myocardial infarction (MI) or from administrative databases of hospital admissions. The study was approved by national and local ethics
committees and written consent was given by all participants. Myocardial infarction was defined according to the recommendation by the European Society of Cardiology/American College of Cardiology Committee (Myocardial infarction redefined 2000). At baseline, diabetes was observed in 19.6% (type 1 diabetes 0.82%). The presence of diabetes was defined by a previous diagnosis of diabetes together with the use of hypoglycemic medication or an elevated HbA1c level at the baseline visit ($\geq 6.4\%$). A total of 1003 survivors of MI were recruited between May 2003 and July 2004. Repeated examination was performed for each subject in six clinical visits every 4 to 6 weeks. Follow-up visits were completed in 86.8% of the participants and the proportion of blood samples taken during the visits ranged from 89.5 to 92.9%. A total of 5794 blood samples from 972 subjects were available for investigation. IL-1Ra was measured in two centres, Helsinki and Augsburg, including 2299 blood samples from 392 individuals. Full details of the study have been described previously (Peters et al. 2007). Baseline data of European MI survivors are shown in Table 7.

Candidate persons for the study were excluded if they were non-resident in the study area, had any major illness preventing them from complying with the study protocol or had chronic significant inflammatory disease and/or anti-inflammatory medication. Recruited subjects were non-smokers or had stopped at least three months before the start of the study.

Each clinic visit was scheduled for the same time of day and the same day of the week to minimize the impact of circadian variation. If a study participant had an acute infection such as a cold or influenza or surgical/dental operation within three days before the scheduled visit, examinations were postponed or blood samples were excluded from the analyses. At each clinic visit a blood sample was drawn for the analysis of inflammatory markers using standardized procedures. Information on smoking, time of the latest meal and health status was collected and a seven-day recall on medication intake was obtained.

4.1.4 FINRISK 97

The FINRISK97 study (FR97) is a population-based risk factor survey with 8444 participants carried out in 5 geographical areas in Finland. The age range was 25 to 74 years (Vartiainen et al. 2010). Baseline characteristics of the study populations are shown in Table 7. The survey was approved by the Ethical Committee of the National
Public Health Institute, and the participants gave a written informed consent. The follow-up of FINRISK 97 included 7 374 subjects aged 25 to 74 years. Baseline characteristics are shown by gender in Table 8. Persons with prevalent diabetes or self-reported impaired glucose tolerance at baseline were excluded from the longitudinal analyses of the present study.

Table 7. Baseline characteristics of myocardial infarction survivors (AIRGENE), Health 2000 study and FINRISK 97 study populations. Data are mean ± standard deviation (SD) unless otherwise indicated.

<table>
<thead>
<tr>
<th></th>
<th>FINRISK 97 n = 7 222</th>
<th>Health 2000 n = 6 771</th>
<th>AIRGENE n = 972</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men, n (%)</td>
<td>3 535 (49.0)</td>
<td>3 011 (44.7)</td>
<td>766 (78.8)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.6 ± 13.4</td>
<td>52.8 ± 16.4</td>
<td>64.6 ± 9.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.6 ± 4.5</td>
<td>26.9 ± 4.9</td>
<td>28.4 ± 4.2</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>1693 (23.5)</td>
<td>1480 (21.8)</td>
<td>75 (7.7)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.54 ± 1.06</td>
<td>5.94 ± 1.12</td>
<td>4.77 ± 1.02</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>136 ± 19</td>
<td>134 ± 34</td>
<td>136 ± 21</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>3587 (49.7)</td>
<td>3173 (46.9)</td>
<td>495 (50.9)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>434 (6.0)</td>
<td>595 (8.8)</td>
<td>191 (19.6)</td>
</tr>
<tr>
<td>Medication for hyperlipidemia, n (%)</td>
<td>249 (3.5)</td>
<td>131 (1.9)</td>
<td>187 (19.2)</td>
</tr>
<tr>
<td>History of stroke, n (%)</td>
<td>162 (2.2)</td>
<td>358 (5.3)</td>
<td>59 (6.1)</td>
</tr>
<tr>
<td>History of MI, n (%)</td>
<td>226 (3.3)</td>
<td>204 (3.0)</td>
<td>972 (100)</td>
</tr>
<tr>
<td>CRP (mg/l)²</td>
<td>1.14 (0.54–2.36)</td>
<td>0.92 (0.40–2.04)</td>
<td>1.37 (0.88–2.60)</td>
</tr>
<tr>
<td>IL-1Ra (μg/l)²</td>
<td>245 (176–324)</td>
<td>326 (223–457)</td>
<td>238 (182–298)</td>
</tr>
</tbody>
</table>

¹Including impaired fasting glucose and impaired glucose tolerance, ²Geometric mean and interquartile range (IQR) are given for variables with skewed distributions

4.1.5 Definitions for glucose abnormalities, metabolic syndrome and type 2 diabetes

Definitions for impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes including previously unknown cases were based on World Health Organization and American Diabetes Association criteria (Report of the Expert Committee 1997, World Health Organization 1999). Thus, diabetes at baseline was defined as fasting plasma glucose (fP-gluc) ≥ 7.0 mmol/l. IFG was diagnosed with fP-gluc ≥ 6.1 mmol/l and < 7.0 mmol/l and IGT was diagnosed after 2 hour post glucose load with P-gluc ≥
7.8 mmol/l. Presence of metabolic syndrome (MetS) was defined by the International Diabetes Federation (IDF) criteria (IDF 2010 http://www.idf.org/webdata/docs/MetS_def_update2006.pdf). These included waist circumference ≥ 94cm and ≥ 80cm for men and women of European origin, respectively, plus any two of the following four factors: 1. raised triglyceride level: ≥ 1.7 mmol/l, or specific treatment, 2. reduced HDL cholesterol: < 1.03 mmol/l in men and < 1.3 mmol/l in women, or medical treatment, 3. systolic blood pressure (bp) ≥ 130 or diastolic bp ≥ 85 mmHg, or treatment for hypertension, 4. fP-gluc ≥ 5.6 mmol/l, or previously diagnosed T2D. Several data sources were used to ascertain exclusion of cases with prevalent diabetes at baseline: (a) self-report of doctor-diagnosed diabetes or impaired glucose tolerance in the questionnaire, (b) the National Drug Reimbursement Register was checked for reimbursements or purchases of hypoglycemic drugs, and (c) the National Hospital Discharge Register was checked for hospitalizations with diabetes as the main or an additional diagnosis, and (d) fasting glucose ≥ 7.0 mmol/l at baseline.

4.1.6 Follow-up for type 2 diabetes

Median follow-up time until the end of 2007 was 10.8 years for the FINRISK 97 and 7.1 years for the Health 2000 cohort. Clinically incident diabetes was the main outcome of interest. Cases of clinically incident diabetes during the follow-up were identified from different data sources. (1) Record linkage with the National Drug Reimbursement Register was on the basis of the personal identification code unique to each individual in the country. (2) Record linkage with the National Hospital Discharge Register, which covers all hospitalizations in the country, was checked for diabetes diagnoses (ICD-10 codes E10-E14) during the follow-up. (3) Record linkage with the National Causes-of-Death Register, which covers all deaths among permanent residents of Finland, was checked for diabetes diagnoses (ICD-10 codes E10-E14) as any cause of death. The date when the diabetes diagnosis first appeared was taken as the date of onset of diabetes. The follow-up procedures identified all diabetes cases treated with hypoglycemic medications or hospitalized or that died during the follow-up. Individuals whose diabetes was treated with diet only and who had no hospitalizations were not identified with these procedures.
Table 8. Characteristics of participants in Health 2000 and FINRISK97 study populations without diabetes at baseline\(^1\). Data are mean ± standard deviation (SD) unless otherwise indicated.

<table>
<thead>
<tr>
<th></th>
<th>Health 2000 men</th>
<th>Health 2000 women</th>
<th>FINRISK97 men</th>
<th>FINRISK 97 women</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2 551</td>
<td>2 960</td>
<td>3 697</td>
<td>3 677</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.9 (11.7)</td>
<td>49.5 (12.2)</td>
<td>49.8 (13.7)</td>
<td>46.9 (12.7)</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>26.9 (4.0)</td>
<td>26.5 (5.0)</td>
<td>26.9 (3.9)</td>
<td>26.1 (4.9)</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>97.2 (11.2)</td>
<td>87.3 (12.9)</td>
<td>94.5 (11.1)</td>
<td>81.2 (11.9)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>742 (29)</td>
<td>564 (19)</td>
<td>1055 (29)</td>
<td>687 (19)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>6.00 (1.12)</td>
<td>5.89 (1.12)</td>
<td>5.56 (1.04)</td>
<td>5.51 (1.07)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.22 (0.33)</td>
<td>1.46 (0.37)</td>
<td>1.26 (0.32)</td>
<td>1.54 (0.35)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.77 (1.20)</td>
<td>1.35 (0.68)</td>
<td>1.67 (1.06)</td>
<td>1.24 (0.81)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>134.3 (18.7)</td>
<td>130.6 (21.2)</td>
<td>139.4 (19.1)</td>
<td>131.7 (19.8)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84.7 (10.7)</td>
<td>80.0 (10.6)</td>
<td>84.5 (11.4)</td>
<td>80.0 (10.8)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>670 (26)</td>
<td>676 (23)</td>
<td>1080 (29)</td>
<td>712 (19)</td>
</tr>
<tr>
<td>Medication for hypertension, n (%)</td>
<td>324 (13)</td>
<td>434 (15)</td>
<td>479 (13)</td>
<td>379 (10)</td>
</tr>
<tr>
<td>Medication for hyperlipidemia, n (%)</td>
<td>141 (6)</td>
<td>141 (5)</td>
<td>161 (4)</td>
<td>55 (2)</td>
</tr>
<tr>
<td>Hormone replacement therapy, n (%)</td>
<td>-</td>
<td>654 (22)</td>
<td>-</td>
<td>590 (16)</td>
</tr>
<tr>
<td>History of CVD event(^2), n (%)</td>
<td>160 (6)</td>
<td>101 (3)</td>
<td>269 (7)</td>
<td>100 (3)</td>
</tr>
<tr>
<td>IL-1Ra (µg/l)(^3)</td>
<td>295 (208–411)</td>
<td>337 (230–473)</td>
<td>225 (167–292)</td>
<td>257 (184–340)</td>
</tr>
<tr>
<td>CRP (mg/l)(^3)</td>
<td>0.52 (0.27–1.72)</td>
<td>0.52 (0.23–1.93)</td>
<td>1.12 (0.53–2.16)</td>
<td>1.07 (0.47–2.32)</td>
</tr>
</tbody>
</table>

\(^1\) Participants with prevalent diabetes (n = 376 for FINRISK97 and 308 for Health 2000) or self-reported impaired glucose tolerance (n = 191 for FINRISK97 and 22 for Health 2000) at baseline were excluded.  
\(^2\) Includes coronary revascularization  
\(^3\) Geometric mean and interquartile range (IQR) are given for variables with skewed distributions.
4.2 GENETIC ANALYSES

4.2.1 Selection and genotyping of IL-1 variants

The study of SAT patients consisted of PCR analysis of genomic DNA that was isolated from mononuclear cells in the peripheral blood samples in 48 patients at the department of Microbiology and Immunology, University of Tampere, Finland. A variable number of tandem repeats (VNTR) polymorphism in the IL-1 receptor antagonist gene (*IL1RN*) was analysed using oligonucleotide primers 5'-CTC AGC AAC ACT CCT AT-3' and 5'-TCC TGG TCT GCA GGT AA-3'. The region which contains the polymorphic site at position -511 of the IL-1 beta (*IL1B*) gene was amplified using oligonucleotide primers 5'-TGG CAT TGA TCT GGT TCA TC-3' and 5'-GTT TAG GAA TCT TCC CAC TT-3'. The tumor necrosis factor alpha (*TNFA*) gene polymorphism was studied using oligonucleotides 5'-AGG CAA TAG GTT TTG AGG GCC AT-3' and 5'-GTT TAG GAA TCT TCC CAC TT-3'.

For genetic analyses, EDTA blood samples were collected and DNA extractions were performed. The isolation of DNA from blood samples in the AIRGENE study was performed in the GSF-National Research Center for Environment and Health in Neuherberg, Germany. Genotyping was performed in the same laboratory at the Institute for Molecular Medicine Finland, Helsinki, Finland for 6 461 individuals in Health 2000, 972 in AIRGENE and 6 052 in the FINRISK 97 study population. Quality control protocols were performed and during aliquotting, pico green fluorescent label (Invitrogen, Molecular Probes, Carlsbad, California, USA) was used to quantify DNA concentration and to normalize the samples for genotyping. We used Sequenom MassARRAY system (Sequenom, San Diego, California, USA) for genotyping SNPs with homogenous mass extension protocol as specified by the manufacturer. In the FINRISK 97 study population, genotyping was also carried out with Sequenom; but it included only 3 SNPs from the IL-1 family in the cross-sectional analyses selected on the basis of the earlier Health 2000 and AIRGENE analyses (original publication III). In the prospective analyses genotyping was performed for 4 972 individuals in Health 2000 and 5 625 in the FINRISK 97 study population. Genotyping of FINRISK 97 study population included four SNPs in the prospective analysis (original publication IV).

Common (> 5% minor allele frequency) haplotype bin tagging SNPs in the IL-1 alpha (*IL1A*), IL-1 beta (*IL1B*) and IL-1 receptor antagonist (*IL1RN*) genes were selected from Seattle SNP variation discovery database based on data available in
September 2005 and are shown in Table 9 (pga.gs.washington.edu). According to the SeattleSNPs database the total number of TagSNPs in the IL-1 family is 102, and our set covers 100 % of the haplotypic variation in IL1A, 100 % in IL1B and 87.5 % in IL1RN with the $r^2$ threshold of 0.65 and minor allele frequency cut-off of 5 %. Selected SNPs in the IL-1 gene family cover 88 (19.2%) of the common variant SNPs in the HapMap data (Phase III, NCBI B36 assembly) region chr2:113259442-113608063 with the $r^2$ threshold of 0.80 (deBakker 2005).

The discordance rate was 0 % in the genotyping sets including 2.2 % known duplicate samples and 2.2% negative controls. Genotyping success rate was more than 95 % for all SNPs. Three SNPs (rs3783546, rs1143629 and rs1143640) were excluded from the analysis due to complete linkage disequilibrium (LD) with rs3783521, rs16944 and rs1143634, respectively ($r^2 > 0.99$): two SNPs represented different haplotype bins in SeattleSNPs database but were in complete LD in our sample and one pair (rs16944 and rs1143629) was purposely selected from the same haplotype bin to ensure the genotyping of IL1B -511C/T. Distribution of genotypes did not deviate from Hardy-Weinberg equilibrium (HWE) except in one case (rs1143642) HWE P-value was 0.014 in the whole population and 0.057 in the OGTT subsample.

4.2.2 Haplotype construction and estimation

Haploview software version 3.32 was used to define haplotype blocks in the IL-1 family (Barret et al. 2005). LD and correlation data plots of IL-1 SNPs are shown in Figure 2. In addition, the algorithm of Gabriel et al. (2002) was used to generate two subhaplotypes from IL1RN (Gabriel et al. 2002). Haplotypes were inferred using PHASE 2.0 software including only individuals with full genotype data (Stephens and Donnelly 2003). Haplotypes were successfully inferred without ambiguous pairs after forty iterations. Haplotypes and haplotype frequencies for the Health 2000 survey population, as well as for the AIRGENE study population, are available within the supplemental data of the original publication II. We also generated subhaplotypes from three SNPs in IL1B by excluding SNP rs1143642 that was found to deviate from HWE. Moreover, the same SNP was found to tag a single haplotype with a carrier frequency of 9.9 %. IL1B subhaplotypes were found to describe the IL1B haplotypes quite well and were used in further analyses.
Table 9. Selected SNPs in the interleukin-1 alpha (IL1A), -beta (IL1B) and receptor antagonist genes (IL1RN) and their minor allele frequencies (MAF) in myocardial infarction survivors (AIRGENE), Health 2000 (H2000) and FINRISK 97 (FR97) populations.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reference SNP ID</th>
<th>Alleles</th>
<th>Location</th>
<th>Seattle SNPs</th>
<th>MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td>literature alias</td>
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<td>0.32</td>
</tr>
</tbody>
</table>

D - deletion, I – insertion, \(^1\) excluded from the further analysis due to complete linkage disequilibrium \((r^2 \geq 0.99)\) with another SNP in the study, \(^2\) included only in the prospective analysis.
Figure 2. LD plots of 15 SNPs from the IL-1α, -β, and receptor antagonist genes using Haploview in the Health 2000 Survey and AIRGENE Population. Haplotype blocks (solid lines) with $r^2$ values are presented. Red Boxes denote Strong LD: $D^* = 1$; Lod Score $\geq 2$. Shades of Pink Red denote significant LD: $D^* < 1$; Lod Score $\geq 2$. White Boxes denote Non-Significant LD: $D^* < 1$; Lod Score $< 2$. 
4.3 LABORATORY METHODS

4.3.1 IL-1Ra and CRP

Plasma levels of IL-1Ra levels in subacute thyroiditis (SAT) patients were measured using an IL-1Ra ELISA-kit (Quantikine, R&D Systems, Inc., MN, USA) at Tampere University Hospital. There were no previous quality control data available for IL-1Ra in the study of SAT patients. CRP was analysed in SAT patients by immunoturbidimetry (normal value below 10 mg/l). The within-assay CV ranged from 1.2 to 0.8 % and between-assay CV from 2.3 to 2.2 %. The erythrocyte sedimentation rate (ESR) was measured by using a 30 min modification of the Westergren method (normal value below 10 mm/h). The within-assay CV is 4.0 % and between-assay CV ranged from 0.0 to 8.5 %.

Plasma levels of IL-1Ra were measured using ELISA in the National Institute for Health and Welfare, Turku, for the AIRGENE study, and in Johannes Gutenberg University, Mainz, Germany, for the Health 2000 and FR97 studies (R&D Systems, Minneapolis, MN, USA). In the AIRGENE study population, the interassay coefficient of variation was 9.0 % at the IL-1Ra concentration of 150 μg/l, 9.7 % at 300 μg/l, 11.5 % at 600 μg/l, and 12.0 % at 1400 μg/l. Mean levels of the 6 follow-up visits were used in the analyses of IL-1Ra concentrations in the AIRGENE study, and coefficients of variations for repeated IL-1Ra measurements ranged from 7.62 to 7.68 % in each visit. Inter-assay and intra-assay CVs for IL-1Ra in Health 2000 and FR97 were 5.68 % and 3.59 %, respectively. CRP was determined using latex immunoassay CRP16 in the National Institute for Health and Welfare, Helsinki, for Health 2000 and FINRISK 97 (Abbott Laboratories, Chicago, IL, USA). Inter-assay and intra-assay CVs were 0.83 % and 0.93 %, respectively.

4.3.2 Glucose and insulin homeostasis

In the Health 2000 survey (n = 6 771) plasma glucose concentration was measured with the hexokinase method using an automated clinical chemistry analyzer after a minimum of 4 h fasting (OlympusAU400, Tokyo, Japan). Inter-assay coefficients of variation for glucose were 2.1 and 2.3 % for mean concentrations of 9.3 and 5.2 mmol/l, respectively. Similar to Health 2000, participants of FR97 were instructed to fast for at least four hours before the scheduled blood drawing and to avoid heavy meals earlier
that day. The median length of fasting was 5 hours (interquartile range 4–6 hours). Plasma insulin concentration was determined with microparticle enzyme immunoassay (Abbott Laboratories, Dainabot, Tokyo, Japan). Inter-assay coefficients of variation for insulin were 4.6 and 4.0% for mean concentrations of 118.7 and 1 032.7 pmol/l, respectively. Hemoglobin A(1c) (HbA1c) was measured with an immunoturbidometric method (Architect ci8200; Abbott Laboratories, Abbott Park, IL). Between-analysis coefficients of variation were 1.8 and 2.0 % for the levels of 5.1 and 10.8 %, respectively. Bias from the laboratory quality reference method was 2.5 % or lower.

In the Health 2000 OGTT subsample (n = 1 390), OGTTs were performed by administering 75 g glucose solution after drawing overnight fasting blood samples for insulin and glucose determinations. Thereafter, blood samples were drawn at 30 and 120 min after the oral glucose ingestion. Plasma glucose concentration was measured by the glucose dehydrogenase method (Diagnostica Merck, Darmstadt, Germany) in an automated clinical chemistry analyzer (Thermo Clinical Labsystems, Konelab, Vantaa, Finland). Inter-assay coefficients of variation for glucose in OGTT were 2.1 and 1.2 % for the mean concentrations of 5.5 and 22.9 mmol/l, respectively. Between-series variation coefficients were 2.0, 1.7, and 1.8 % for the mean concentrations of 2.1, 5.1, and 15.3 mmol/l, respectively. Plasma insulin concentration was measured with a RIA kit (Phadeseph insulin RIA; Pharmacia, Uppsala, Sweden). Coefficients of variation for plasma insulin were as follows: within series, 5.2 and 3.6 % for the concentrations below and above 138.9 pmol/l respectively, and between series, 5.7 and 6.3 % for the mean concentrations 94.4 and 745.9 pmol/l respectively.

Insulin sensitivity and pancreatic β-cell function were analyzed by using the homeostasis model assessment of insulin resistance (HOMA-IR) and beta-cell function (HOMA-β-cell). Formulas for HOMA models are: HOMA-IR = (fP-insulin pmol/l × fP-glucose mmol/ l)/22.5; HOMA-β-cell = 20 × fP-insulin/(fP-glucose - 3.5), where fP-insulin (pmol/l) and fP-glucose (mmol/l) are mean plasma insulin and glucose, respectively (SI unit correction for insulin: pmol/l = 6.945 × μIU/ml).
4.3.3 Other laboratory methods
Lipid measurements, including serum total cholesterol, HDL cholesterol and triglycerides were performed by using routine enzymatic methods and quality assurance. For the Health 2000 survey, the determinations were carried out at the Social Insurance Institution’s Research and Development Unit in Turku and for FINRISK 97 at the Laboratory of Analytical Biochemistry of the National Public Health Institute in Helsinki. Enzymatic methods were used with cholesterol oxidase for total cholesterol measurement and glycerol-3-phosphate oxidase for triglyceride measurement. HDL cholesterol was measured by using direct enzymatic methods. The quality of the results of the series of analysis was ascertained by using controls, which were used to determine interassay coefficients of variation. The laboratories took part in Labquality’s External Quality Assessment Schemes and in the quality control programmes led by the Centers of Disease Control and Prevention and the National Heart Lung and Blood Institute, USA. In patients with SAT, thyroid antibodies were measured by a specific IgG fluoroenzymeimmunoassay (FEIA) UniCAP for thyroglobulin (TG) antibodies and for thyroid peroxidase (TPO) antibodies (Pharmacia&Upjohn). The cut-off value for TG antibodies was 344 IU/ml, with a detection limit of 172 IU/ml indicating expression. For TPO antibodies the cut-off value was 100 IU/ml, with a detection limit of 20 IU/ml. Within-assay CV was 2.6 % and between-assay 3.8 % for TPO antibodies. The corresponding values for TG antibodies were 4.8 % and 3.9 %. Serum levels of free thyroxin were measured by fluoroimmunoassay (normal range 9.0-19.0 pmol/l) and thyrotropin by immunofluorometric analysis (normal range from 0.40 to 6.0 mU/l).

4.4 STATISTICAL METHODS
4.4.1 Inflammatory gene variations, thyroid antibodies and subacute thyroiditis
Frequency (number of test alleles divided by total number of alleles expressed as percentage) and carriage rate (percentage of individuals carrying at least one copy of the test allele) of IL1RN VNTR allele 2, IL-1β-511 SNP allele 2 and TNFα SNP allele 2 were calculated, and their association with thyroid antibodies was studied using the chi-square test. Pearson’s correlation coefficients and their P-values were used for analysing the dependencies between the IL1-Ra levels, thyroid antibodies and acute-phase reactants.
4.4.2 IL-1 gene variation, IL-1Ra phenotypes and nongenetic traits
Interleukin-1 receptor antagonist was log-transformed, and geometric means were reported. Linear regression analysis based on additive models was used for the multivariate analyses and adjusted for age, sex, body mass index (BMI), total cholesterol to high-density lipoprotein (HDL) cholesterol ratio, systolic blood pressure, current smoking, hypertension, and residential area. Trends across the 3 genotypes were computed including effect per allele estimates. Proportions of IL-1Ra phenotypic variance explained by genetic and nongenetic factors were calculated by using formula: $SS_{\text{eff}} / SS_{\text{tot}}$ ($SS = \text{sum of squares, eff = effect variable, tot = whole model}$). Replication of the results was evaluated in the 3 study populations, and the results were pooled using fixed-effect meta-analysis.

4.4.3 Type 2 diabetes, glucose homeostasis and IL-1 variation
The parameters for plasma glucose and insulin homeostasis were log-transformed for the analysis to satisfy the normality assumptions, whereas geometric means are reported. Covariates in the multivariate models were age, gender, body mass index (BMI), waist circumference, mean systolic blood pressure, smoking, alcohol consumption, level of education, and area of residence. We tested for association using a general test with two degrees of freedom, where the heterozygote and minor homozygote genotypes were considered as two dichotomous variables and the major homozygote genotype as the reference category. Trends across the three genotypes were also analyzed with linear regression analysis using an additive model and we mainly report the $p$ values from the trend tests. Poisson regression analysis was used to examine the association of IL-1 gene variation with type 2 diabetes in the Health 2000 and AIRGENE study populations. The analyses of glucose homeostasis were carried out for individuals without hypoglycaemic medication. The adjusted mean levels of the glucose homeostasis traits by genotypes were computed by using General Linear Models. For the OGTT subsample, we computed an area under the glucose curve (AUC glucose, mmol/l × min) and area under the insulin curve (AUC insulin, mU/l × min) according to the trapezium rule as summary measures for the glucose and insulin responses by each genotype.
4.4.4 Adjusting for multiple testing and power calculations
Bonferroni correction was used in cross-sectional analyses to adjust for multiple testing, but nominal p-values are also shown. Haplotypes were correspondingly analyzed by comparing the carriers of 2 copies of the haplotype with the carriers of 1 copy or no copy of the haplotype (or simple carrier analysis). Bonferroni correction was not done for the haplotype analyses. Statistical analyses were carried out with SAS, version 9.1.3 (SAS Institute, Cary, NC). Power calculations for the haplotype association analyses with prevalent diabetes were carried out with the Power for Genetic Association Analyses package and are depicted in the supplemental material of the original publication II (Menashe et al. 2008).

4.4.5 Prospective analysis for incident type 2 diabetes
Health 2000 and FINRISK 1997 study populations were divided into three gender-specific groups that were nondiabetic subjects without MetS at baseline, subjects with MetS but no diabetes at baseline, and those with diabetes at baseline. Differences in IL-1Ra and CRP concentrations were compared between these groups with ANOVA adjusting for age. Cox proportional hazards regression models were used for prospective analyses to examine different factors predicting clinically incident diabetes. Trends across the three genotypes were reported as effect per allele estimates and variants in the IL-1 family were analyzed using additive models unless otherwise indicated, adjusted for age, BMI, systolic blood pressure, total cholesterol, current smoking (yes -1, no - 0), area of residence (east vs. west), alcohol consumption (intake frequency categories in FINRISK 97 from 1 to 3 and in Health 2000 from 1 to 6), education (levels: basic - 1, middle - 2 and upper - 3), baseline history of cardiovascular disease (CVD) event (yes - 1, no - 0) and IL-1Ra. Alternatively, the model included waist circumference, hypertension and total cholesterol to HDL cholesterol ratio instead of total cholesterol. Analyses were run with and without CRP and blood glucose in the model. Data of the two cohorts were pooled for the prospective analyses. FINRISK 97 had 33.5 % of missing data for baseline plasma glucose. These were replaced with the mean blood glucose. Health 2000 had no missing data for the baseline plasma glucose. The predictive power of IL-1Ra and CRP for incident diabetes was analyzed in subjects with MetS defined by IDF criteria but no diabetes at the beginning of the follow-up.
4.5 ETHICAL CONSIDERATIONS

All projects have been approved by their respective hospital ethics committees, and the participants gave a written informed consent. The studies were carried out according to the recommendations of the Declaration of Helsinki. The population based and multi-centre studies were additionally approved by institutional and national ethics committees.
5. RESULTS

5.1 IL-1 VARIATION AND THYROID ANTIBODIES IN SUBACUTE THYROIDITIS

5.1.1 IL-1 family gene variants, TPO antibodies and acute inflammation

Measurable levels of TPO or thyroglobulin (TG) antibodies were found in 26.4 % and 37.7 % of patients, respectively. The clinically positive thyroid antibodies exceeding the cut-off values were found in 13.2 % of patients for TPO antibodies and 15.1 % of patients for TG antibodies. Positive levels of TPO antibodies were observed only in women (P = 0.041). The levels of IL-1Ra were positively correlated with CRP (\(r = 0.409, P = 0.004\)) and ESR (\(r = 0.346, P = 0.016\)) (original publication I). Age, sex and the duration of the symptoms did not show any associations with the IL-1Ra levels. TPO antibody levels were negatively correlated with CRP (\(r = -0.272, P = 0.046\)) and ESR (\(r = -0.322, P = 0.017\)). CRP levels were higher in men as compared to women (76.9 ± 37.2 vs. 44.6 ± 37.4, P = 0.007) and no associations were found with age or the duration of the symptoms. IL-1Ra levels were not significantly associated with the genotypes, although a decreasing trend was seen for simultaneous minor allele of \(IL1RN\) and \(IL1B\) as well as in subjects with positive levels of TPO antibodies at baseline (original publication I).

Carriage of the minor allele of the \(IL1RN\) VNTR polymorphism (IL1RN*2) was associated with measurable levels of thyroid peroxidase (TPO) antibodies as shown in Table 10. The simultaneous carriage of IL1RN*2 and the minor allele of \(IL1B\) at position -511 (rs16944) was also associated with measurable levels of TPO antibodies in patients with subacute thyroiditis (SAT) while the minor variant of \(IL1B\) rs16944 alone showed only a trend towards an association. Likewise, IL1RN*2 frequency was associated with measurable levels of TPO antibodies (P = 0.039) (original publication I). The studied inflammatory gene polymorphisms showed no significant differences in genotypic distributions between the healthy blood donors and SAT patients (original publication I).

5.1.2 Follow-up for subacute thyroiditis patients

The follow-up examination included 48 individuals out of 54 subjects that had been studied at baseline, after 2.4 years (mean, range 0.7 – 3.8). Persistent hypothyroidism after SAT was diagnosed in four (8.3 %) individuals as defined by altered serum levels
of thyrotropin, free thyroxine or ongoing thyroxine medication as shown in Table 11 (unpublished data). The presence of measurable amounts of thyroid microsomal antibodies and hypothyroidism was found in three cases. Overall, measurable amounts of thyroid microsomal antibodies were found in eight (16.7 %) subjects at the follow-up. The simultaneous presence of at least one minor allele for \textit{IL1RN} VNTR and \textit{IL1B} rs16944 was found in all subjects with the diagnosis of persistent hypothyroidism following SAT at the follow-up visit.

Table 10. Carriage of the rare alleles of \textit{IL1RN} VNTR (\textit{IL1RN}*-2), \textit{IL1B} SNP at position -511 [rs16944] (\textit{IL1B}-511*-2) and \textit{TNFA} (\textit{TNFA}*2) according to existence of TPO antibodies (TPOab) and thyroglobulin antibodies (TGab) tested in 48 subjects with subacute thyroiditis.

<table>
<thead>
<tr>
<th>alleles</th>
<th>Measurable TPOab</th>
<th>no TPOab</th>
<th>P</th>
<th>Measurable TGab</th>
<th>no TGab</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{IL1RN}*-2</td>
<td>11 (79%)</td>
<td>14 (41%)</td>
<td>0.018</td>
<td>12 (60%)</td>
<td>13 (46%)</td>
<td>0.35</td>
</tr>
<tr>
<td>\textit{IL1B}-511*-2</td>
<td>12 (86%)</td>
<td>21 (62%)</td>
<td>0.097</td>
<td>16 (80%)</td>
<td>17 (61%)</td>
<td>0.16</td>
</tr>
<tr>
<td>\textit{IL1RN}<em>-2 and \textit{IL1B}-511</em>-2</td>
<td>10 (71%)</td>
<td>9 (27%)</td>
<td>0.0038</td>
<td>9 (45%)</td>
<td>10 (36%)</td>
<td>0.52</td>
</tr>
<tr>
<td>\textit{TNFA}*2</td>
<td>4 (29%)</td>
<td>14 (41%)</td>
<td>0.41</td>
<td>7 (35%)</td>
<td>11 (39%)</td>
<td>0.76</td>
</tr>
<tr>
<td>total n = 48</td>
<td>14</td>
<td>34</td>
<td>0.41</td>
<td>20</td>
<td>28</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Measurable TPOab was considered at a serum concentration over 20 IU/ml and TGab over 172 IU/ml.
Table 11. Data from thirteen female subacute thyroiditis patients with the presence of thyroid antibodies or hypothyroidism at follow-up examination. Subjects carrying the minor allele of *IL1RN* VNTR or *IL1B* SNP at position -511 (rs16944) are shown.

<table>
<thead>
<tr>
<th>VNTR/ rs16944 minor allele</th>
<th>age years</th>
<th>steroid therapy</th>
<th>fine-needle cytology</th>
<th>TPOab (IU/ml)</th>
<th>TMab (titre)</th>
<th>TGab (IU/ml)</th>
<th>TPOab (IU/ml)</th>
<th>TMab (titre)</th>
<th>TGab (IU/ml)</th>
<th>hypothyroidism</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/-</td>
<td>35</td>
<td>no</td>
<td>giant cells</td>
<td>1435</td>
<td>1:25600</td>
<td>2295</td>
<td>2</td>
<td>55</td>
<td>1:25600</td>
<td>298</td>
</tr>
<tr>
<td>+/-</td>
<td>47</td>
<td>yes</td>
<td>lymphocyte</td>
<td>910</td>
<td>1:6400</td>
<td>1022</td>
<td>3.6</td>
<td>&lt; 20</td>
<td>1:400</td>
<td>&lt; 172</td>
</tr>
<tr>
<td>-/-</td>
<td>79</td>
<td>yes</td>
<td>giant cells</td>
<td>773</td>
<td>1:400</td>
<td>&lt; 172</td>
<td>1.3</td>
<td>&lt; 20</td>
<td>1:6400</td>
<td>&lt; 172</td>
</tr>
<tr>
<td>+/-</td>
<td>62</td>
<td>no</td>
<td>lymphocyte</td>
<td>325</td>
<td>1:6400</td>
<td>&lt; 172</td>
<td>1.6</td>
<td>31</td>
<td>1:6400</td>
<td>&lt; 172</td>
</tr>
<tr>
<td>+/-</td>
<td>51</td>
<td>yes</td>
<td>lymphocyte</td>
<td>247</td>
<td>1:1600</td>
<td>430</td>
<td>0.8</td>
<td>117</td>
<td>1:1600</td>
<td>&lt; 172</td>
</tr>
<tr>
<td>+/-</td>
<td>48</td>
<td>yes</td>
<td>insufficient</td>
<td>243</td>
<td>1:400</td>
<td>&lt; 172</td>
<td>3</td>
<td>24</td>
<td>1:1600</td>
<td>&lt; 172</td>
</tr>
<tr>
<td>+/-</td>
<td>51</td>
<td>no</td>
<td>giant cells</td>
<td>164</td>
<td>1:6400</td>
<td>219</td>
<td>1.5</td>
<td>29</td>
<td>1:6400</td>
<td>&lt; 172</td>
</tr>
<tr>
<td>+/-</td>
<td>53</td>
<td>no</td>
<td>giant cells</td>
<td>&lt; 20</td>
<td>0</td>
<td>2259</td>
<td>1.25</td>
<td>&lt; 20</td>
<td>0</td>
<td>&lt; 172</td>
</tr>
<tr>
<td>+/-</td>
<td>50</td>
<td>yes</td>
<td>insufficient</td>
<td>23</td>
<td>0</td>
<td>364</td>
<td>1.1</td>
<td>&lt; 20</td>
<td>0</td>
<td>&lt; 172</td>
</tr>
<tr>
<td>-/+</td>
<td>72</td>
<td>yes</td>
<td>giant cells</td>
<td>65</td>
<td>1:400</td>
<td>5002</td>
<td>1.8</td>
<td>&lt; 20</td>
<td>1:400</td>
<td>177</td>
</tr>
<tr>
<td>+/-</td>
<td>41</td>
<td>yes</td>
<td>giant cells</td>
<td>55</td>
<td>0</td>
<td>923</td>
<td>1.1</td>
<td>&lt; 20</td>
<td>0</td>
<td>&lt; 172</td>
</tr>
<tr>
<td>+/-</td>
<td>56</td>
<td>yes</td>
<td>giant cells</td>
<td>&lt; 20</td>
<td>0</td>
<td>&lt; 172</td>
<td>3.3</td>
<td>&lt; 20</td>
<td>0</td>
<td>&lt; 172</td>
</tr>
<tr>
<td>+/-</td>
<td>68</td>
<td>yes</td>
<td>insufficient</td>
<td>&lt; 20</td>
<td>0</td>
<td>&lt; 172</td>
<td>2.4</td>
<td>&lt; 20</td>
<td>0</td>
<td>&lt; 172</td>
</tr>
</tbody>
</table>

Thyroid peroxidase antibodies (TPOab), Thyroid microsomal antibodies (TMab), Thyroglobulin antibodies (TGab)

\(^1\)Graves’ disease developed

\(^2\)previous surgery for multinodular goiter
5.2 IL-1 VARIATION, IL-1RA AND GLUCOSE HOMEOSTASIS

5.2.1 IL1A variation

The promoter IL1A variant rs1800587 was nominally associated with the increased risk for clinically incident diabetes amongst men in Health 2000, as shown by the increment of the risk per number of the minor alleles or analysis using the dominant model (Table 12). Amongst women, the association was also significant but in the opposite direction. A significant interaction by gender was also found for IL1A rs1800587 (P = 0.002 for gender by genotype interaction). Rs1800587 was not genotyped in FR97, thus replication of the interaction is not known. The exclusion of IL-1R as a covariate was followed by a slight attenuation of the hazard ratio and significance level in men (original publication IV). The further adjustment for CRP concentrations did not affect the associations between diabetes and genetic markers. Rs1800587 was nominally associated with higher plasma glucose in the cross-sectional analyses (Table 13). Additionally, rs1800587 was nominally associated with higher IL-1Ra concentration in Health 2000 (Table 14).

The minor allele of IL1A rs2856838 was associated with decreased HbA1c and glucose levels in Health 2000 (Table 13) while in the OGTT subsample analysis the results remained significant after Bonferroni correction (original publication II supplemental data). Rs2856838 was also associated with a trend towards decreased IL-1Ra levels in the healthy population as shown in Table 14. The minor allele of IL1A rs3783548 with MAF \( \leq 0.1 \) was associated with decreased IL-1Ra levels in MI survivors and in the Health 2000 population. Rs3783548 was associated with increased risk for clinically incident diabetes in men and had a trend towards increased HbA1c levels (Tables 12 and 13).
Table 12. Hazard ratios (HR) with 95% confidence intervals (CI 95%) of SNPs in genes *IL1A*, *IL1B* and *IL1RN* for clinically incident diabetes.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Sex</th>
<th>Beta</th>
<th>StdErr</th>
<th>HR (CI 95%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1800587</td>
<td>M</td>
<td>0.405</td>
<td>0.16</td>
<td>1.50 (1.10 – 2.05)</td>
<td>0.011</td>
</tr>
<tr>
<td>rs1800587</td>
<td>W</td>
<td>-0.379</td>
<td>0.188</td>
<td>0.68 (0.47 – 0.99)</td>
<td>0.044</td>
</tr>
<tr>
<td>rs1143634</td>
<td>M</td>
<td>0.371</td>
<td>0.164</td>
<td>1.50 (1.05 – 2.00)</td>
<td>0.024</td>
</tr>
<tr>
<td>rs1143634</td>
<td>W</td>
<td>-0.147</td>
<td>0.192</td>
<td>0.86 (0.59 – 1.26)</td>
<td>0.443</td>
</tr>
<tr>
<td>rs3213448</td>
<td>M</td>
<td>0.333</td>
<td>0.172</td>
<td>1.40 (1.00 – 1.95)</td>
<td>0.053</td>
</tr>
<tr>
<td>rs3213448</td>
<td>W</td>
<td>-0.1</td>
<td>0.192</td>
<td>0.90 (0.62 – 1.32)</td>
<td>0.603</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SNP</th>
<th>Sex</th>
<th>Beta</th>
<th>StdErr</th>
<th>HR (CI 95%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1800587</td>
<td>M</td>
<td>0.835</td>
<td>0.289</td>
<td>2.30 (1.31 – 4.06)</td>
<td>0.004</td>
</tr>
<tr>
<td>rs1800587</td>
<td>W</td>
<td>-0.179</td>
<td>0.401</td>
<td>0.834 (0.38 – 1.84)</td>
<td>0.655</td>
</tr>
<tr>
<td>rs3783548</td>
<td>M</td>
<td>1.457</td>
<td>0.726</td>
<td>4.29 (1.03 – 17.82)</td>
<td>0.045</td>
</tr>
<tr>
<td>rs3783548</td>
<td>W</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>rs1143642</td>
<td>M</td>
<td>0.768</td>
<td>1.015</td>
<td>2.16 (0.29 – 15.77)</td>
<td>0.449</td>
</tr>
<tr>
<td>rs1143642</td>
<td>W</td>
<td>0.692</td>
<td>1.017</td>
<td>2.00 (0.27 – 14.65)</td>
<td>0.496</td>
</tr>
<tr>
<td>rs1143634</td>
<td>M</td>
<td>0.485</td>
<td>0.372</td>
<td>1.63 (0.78 – 3.37)</td>
<td>0.192</td>
</tr>
<tr>
<td>rs1143634</td>
<td>W</td>
<td>-0.265</td>
<td>0.517</td>
<td>0.77 (0.28 – 2.11)</td>
<td>0.608</td>
</tr>
<tr>
<td>rs3213448</td>
<td>M</td>
<td>0.707</td>
<td>0.385</td>
<td>2.028 (0.95 – 4.32)</td>
<td>0.067</td>
</tr>
<tr>
<td>rs3213448</td>
<td>W</td>
<td>-0.14</td>
<td>0.518</td>
<td>0.87 (0.31 – 2.4)</td>
<td>0.787</td>
</tr>
<tr>
<td>rs315949</td>
<td>M</td>
<td>-1.115</td>
<td>0.589</td>
<td>0.33 (0.10 – 1.04)</td>
<td>0.059</td>
</tr>
<tr>
<td>rs315949</td>
<td>W</td>
<td>-0.433</td>
<td>0.465</td>
<td>0.65 (0.26 – 1.61)</td>
<td>0.352</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SNP</th>
<th>Sex</th>
<th>Beta</th>
<th>StdErr</th>
<th>HR (CI 95%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1800587</td>
<td>M</td>
<td>0.355</td>
<td>0.221</td>
<td>1.43 (0.92 – 2.20)</td>
<td>0.109</td>
</tr>
<tr>
<td>rs1800587</td>
<td>W</td>
<td>-0.548</td>
<td>0.231</td>
<td>0.58 (0.37 – 0.91)</td>
<td>0.018</td>
</tr>
<tr>
<td>rs1143634</td>
<td>M</td>
<td>0.465</td>
<td>0.217</td>
<td>1.59 (1.04 – 2.44)</td>
<td>0.032</td>
</tr>
<tr>
<td>rs1143634</td>
<td>W</td>
<td>-0.158</td>
<td>0.23</td>
<td>0.85 (0.54 – 1.34)</td>
<td>0.493</td>
</tr>
<tr>
<td>rs3213448</td>
<td>M</td>
<td>0.327</td>
<td>0.217</td>
<td>1.97 (0.91 – 2.12)</td>
<td>0.131</td>
</tr>
<tr>
<td>rs3213448</td>
<td>W</td>
<td>-0.116</td>
<td>0.23</td>
<td>0.89 (0.57 – 1.40)</td>
<td>0.613</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SNP</th>
<th>Sex</th>
<th>Beta</th>
<th>StdErr</th>
<th>HR (CI 95%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1800587</td>
<td>M</td>
<td>0.003</td>
<td>0.112</td>
<td>1.00 (0.81 – 1.25)</td>
<td>0.98</td>
</tr>
<tr>
<td>rs1800587</td>
<td>W</td>
<td>-0.279</td>
<td>0.15</td>
<td>0.76 (0.56 – 1.01)</td>
<td>0.063</td>
</tr>
<tr>
<td>rs1143634</td>
<td>M</td>
<td>-0.138</td>
<td>0.134</td>
<td>0.87 (0.67 – 1.13)</td>
<td>0.304</td>
</tr>
<tr>
<td>rs1143634</td>
<td>W</td>
<td>-0.2</td>
<td>0.158</td>
<td>0.82 (0.60 – 1.12)</td>
<td>0.207</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SNP</th>
<th>Sex</th>
<th>Beta</th>
<th>StdErr</th>
<th>HR (CI 95%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1800587</td>
<td>M</td>
<td>0.066</td>
<td>0.143</td>
<td>1.07 (0.81 – 1.41)</td>
<td>0.646</td>
</tr>
<tr>
<td>rs1800587</td>
<td>W</td>
<td>-0.328</td>
<td>0.179</td>
<td>0.72 (0.51 – 1.02)</td>
<td>0.067</td>
</tr>
<tr>
<td>rs1143634</td>
<td>M</td>
<td>-0.108</td>
<td>0.163</td>
<td>0.90 (0.65 – 1.23)</td>
<td>0.505</td>
</tr>
<tr>
<td>rs1143634</td>
<td>W</td>
<td>-0.294</td>
<td>0.188</td>
<td>0.74 (0.52 – 1.08)</td>
<td>0.118</td>
</tr>
</tbody>
</table>

Adjusted for age, BMI, systolic blood pressure, total cholesterol to HDL cholesterol ratio, smoking (0, 1), area of residence (east vs. west), waist circumference, alcohol consumption (from 1 to 6 in Health 2000 and 1 to 3 in FR97), hypertension (0,1), education (from 1 to 3), baseline CVD (0, 1) event and IL-1Ra.
5.2.2 IL1B variation

The minor allele of the coding \textit{IL1B} rs1143634 was nominally associated with increased risk of clinically incident diabetes as shown by the increment of risk per number of the minor allele or the analysis with dominant model in Health 2000 men (Table 12). Amongst women, the association tended to be in the opposite direction but was nonsignificant. In FR97, no significant associations were observed, but when both cohorts were combined, rs1143634 was nominally associated with reduced risk for diabetes amongst women. A marginally significant SNP by gender interaction was observed for rs1143634 in Health 2000 ($P = 0.047$) and the interaction strengthened when the Health 2000 and FR97 cohorts were pooled ($P = 0.023$). The exclusion of IL-1Ra or HDL cholesterol among the covariates in the multivariate analysis was followed by a slight attenuation of the hazard ratio and significance level in men (original publication IV). Adjustment for CRP was followed by a slight increase of the significance level.

The minor allele of \textit{IL1B} rs1143634 polymorphism was associated with higher glucose levels than the major allele: 5.37, 5.41, and 5.48 mmol/l for the GG ($n = 3319$), AG ($n = 2276$) and AA ($n = 415$) genotypes, respectively (Bonferroni corrected $P$ for trend = 9.6e-4). As shown in Table 13, rs1143634 was associated with plasma glucose and HbA1c levels in Health 2000. The association of rs1143634 minor allele and increased HbA1c remained significant after Bonferroni correction only in the OGTT subsample as shown in the supplemental data of the original publication II (Bonferroni corrected $P$ for trend = 0.0075). Additionally, plasma 2 hour glucose levels in OGTT were nominally increased in rs1143634 minor allele homozygotes. Nominal significant association with increased IL-1Ra levels was found for rs1143634 in Health 2000 as shown in Table 14.

The large-scale replication data for susceptibility loci for T2D was sought \textit{in silico} from several genome wide association databases available and the results were compared to the present data for the associations with metabolic traits (original publication II). Five SNPs were previously associated with insulin, 2 hour insulin or HOMA-IR and were in high LD with rs1143634 ($r^2 > 0.80$). The direction of the associations was consistent with the present study. Furthermore, three SNPs were in high LD with rs1143634 ($r^2 > 0.80$) and they associated with diabetes in the direction, which was consistent with our findings of plasma glucose levels.
The individuals carrying two copies of the *IL1B* haplotype ACG (rs1143634, rs3917356 and rs16944) in Health 2000 had a higher prevalence for diabetes when compared with those not carrying the haplotype or carrying only one copy of the haplotype [risk ratio (RR) (95%CI) 1.54 (1.03 – 2.30), P = 0.037] (original publication II). Correspondingly, the ACG haplotype was consistently associated with prevalent diabetes in European myocardial infarction survivors [RR (CI95%) 2.09 (1.17 – 3.76), P = 0.014]. Individuals with type 1 diabetes were excluded from the analyses shown but including them did not change the results. In the prospective analyses, the ACG haplotype did not show any significant association, whereas the haplotype ATG was likewise associated with increased risk amongst men both in the additive analysis [HR (95%CI) 2.20 (11.80 – 4.08), P = 0.013] and in the carrier analysis [HR (CI95%) 2.38 (1.25 – 4.51), P = 0.008] (original publication IV). Both these *IL1B* haplotypes are tagged by rs1143634.

The increasing number of the *IL1B* haplotype copies of ACG (rs1143634, rs3917356 and rs16944) was associated with higher plasma glucose (P for trend = 0.0003), insulin (P for trend = 0.049) and HOMA-IR (P for trend = 0.015). As also reported in the original publication II, the number of *IL1B* ACG haplotype copies was associated with higher AUC glucose in OGTT analysis (6.805, 6.809 and 6.903 for persons with 0, 1 and 2 copies of the haplotype, respectively, P for trend = 0.015) as well as with glucose and insulin measurements at the different time points (Table 15).

The minor allele of the intronic *IL1B* rs1143642 with MAF ≤ 0.07 was associated with the increased risk for clinically incident diabetes in FINRISK 97 women in the dominant model analysis (Table 12). The direction of the association was similar in Health 2000, although not statistically significant. In both study populations combined, the risk estimates for rs1143642 approached statistical significance in women. In men, rs1143642 was not significantly associated with the increased risk for clinically incident diabetes. The only difference that remained significant after correction for multiple testing in OGTT was the 2 hour insulin value for the *IL-1B* rs1143642, where the minor allele was associated with lower plasma insulin (original publication II). The rare allele of rs1143642 was associated with decreased IL-1Ra levels in Health 2000 and FINRISK 97 populations after Bonferroni correction in the meta-analysis (Table 14).
5.2.3 IL1RN variation

The minor allele of *IL1RN* rs3213448 was associated with increased IL-1Ra levels after Bonferroni correction in the meta-analysis, and the direction of the association was consistent in 3 study populations as shown in Table 14. A trend towards the increased risk for the development of diabetes was found in the prospective analysis in Health 2000 men, but no association was found in men in FINRISK 97, or in women of either cohort (Table 12). The associations with insulin levels in OGTT were nominally significant for *IL1RN* rs3213448 (original publication II).

The minor allele of *IL1RN* rs315949 approached statistical significance for protection from diabetes in Health 2000 men using the dominant model (Table 12). Rs315949 was also robustly associated with lower IL-1Ra levels (Table 14). Furthermore, Rs315949 is known to be in strong linkage disequilibrium ($r^2 = 0.84$) with rs4251961 (Rafiq et al. 2007), the minor allele of which was more frequent in the group with a better response to anakinra in the treatment of type 2 diabetes (Larsen et al. 2009). In our study, rs315949 was genotyped in 2 populations (Table 14); and its minor allele was robustly associated with decreased IL-1Ra level in both populations showing a significant association in meta-analysis after the Bonferroni correction.

The minor allele of *IL1RN* rs4252041 (MAF ≤ 0.041) was associated with increased HbA1c levels in the OGTT subsample remaining significant after the Bonferroni correction (original publication II supplemental data). Rs4252041 was robustly associated with decreased IL-1Ra levels only among MI survivors as shown in Table 14. The synonymous coding minor allele of *IL1RN* rs315952 was robustly associated with increased IL-1Ra levels in 2 cohorts and meta-analyses. The *IL1RN* rs2637988 was robustly associated with the IL-1Ra phenotype in Health 2000 population and meta-analysis. The haplotypic variation of *IL1RN* including SNPs rs315952, rs4252041 and rs315949 was associated with IL-1Ra levels as shown in Table 16.
Table 13. Associations of the SNPs (additive model) in the interleukin-1 gene family with plasma glucose, insulin and glycosylated hemoglobin A(1c) (HbA1c) in the Health 2000\(^1\) study.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Glucose (mmol/l)</th>
<th>Beta(^2)</th>
<th>P</th>
<th>Insulin (pmol/l)</th>
<th>Beta(^2)</th>
<th>P</th>
<th>HbA1c (%)</th>
<th>Beta(^2)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL1A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3783521</td>
<td>-0.007</td>
<td>0.78</td>
<td></td>
<td>-0.061</td>
<td>0.49</td>
<td></td>
<td>0.002</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>rs1800587</td>
<td>0.030</td>
<td>0.025</td>
<td></td>
<td>0.084</td>
<td>0.46</td>
<td></td>
<td>0.001</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>rs2856838</td>
<td>-0.015</td>
<td>0.045</td>
<td></td>
<td>-0.013</td>
<td>0.98</td>
<td></td>
<td>-0.004</td>
<td>0.007(^5)</td>
<td></td>
</tr>
<tr>
<td>rs3783548(^3)</td>
<td>-0.010</td>
<td>0.33</td>
<td></td>
<td>-0.023</td>
<td>0.68</td>
<td></td>
<td>0.004</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td><strong>IL1B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs16944</td>
<td>-0.018</td>
<td>0.19</td>
<td></td>
<td>-0.039</td>
<td>0.53</td>
<td></td>
<td>-0.002</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>rs3917356</td>
<td>-0.013</td>
<td>0.13</td>
<td></td>
<td>-0.042</td>
<td>0.29</td>
<td></td>
<td>-0.0002</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>rs1143634</td>
<td>0.056</td>
<td>&lt;0.0001(^4)</td>
<td></td>
<td>0.139</td>
<td>0.19</td>
<td></td>
<td>0.004</td>
<td>0.008(^5)</td>
<td></td>
</tr>
<tr>
<td>rs1143642(^3)</td>
<td>-0.002</td>
<td>0.86</td>
<td></td>
<td>0.0004</td>
<td>0.10</td>
<td></td>
<td>-0.003</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td><strong>IL1RN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2637988</td>
<td>-0.015</td>
<td>0.19</td>
<td></td>
<td>-0.063</td>
<td>0.37</td>
<td></td>
<td>-0.001</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>rs3213448</td>
<td>0.019</td>
<td>0.10</td>
<td></td>
<td>-0.017</td>
<td>0.99</td>
<td></td>
<td>0.001</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>rs315934(^3)</td>
<td>-0.011</td>
<td>0.19</td>
<td></td>
<td>-0.083</td>
<td>0.093</td>
<td></td>
<td>0.001</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>rs4252008</td>
<td>-0.009</td>
<td>0.88</td>
<td></td>
<td>-0.035</td>
<td>0.26</td>
<td></td>
<td>-0.001</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>rs315952</td>
<td>0.008</td>
<td>0.54</td>
<td></td>
<td>-0.030</td>
<td>0.95</td>
<td></td>
<td>0.002</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>rs4252041(^3)</td>
<td>0.031</td>
<td>0.13</td>
<td></td>
<td>0.180</td>
<td>0.14</td>
<td></td>
<td>0.008</td>
<td>0.093</td>
<td></td>
</tr>
<tr>
<td>rs315949</td>
<td>-0.012</td>
<td>0.33</td>
<td></td>
<td>-0.088</td>
<td>0.79</td>
<td></td>
<td>-0.001</td>
<td>0.51</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Adjusted for age, gender, BMI, waist circumference, mean systolic blood pressure, smoking, alcohol consumption, level of education and area of residence.

\(^2\)\(\hat{\beta}\) calculated per increasing copy of minor allele, coded as 0, 1, 2.

\(^3\)Minor allele homozygotes and heterozygotes pooled, because the prevalence of minor allele homozygotes is less than 5%.

\(^4\)Bonferroni corrected P-value 0.00096.

\(^5\)Bonferroni corrected P-values 0.10 and 0.12.
Table 14. The multivariate-adjusted associations of SNPs in interleukin-1 alfa (IL1A) interleukin-1 beta (IL1B) and receptor antagonist (IL1RN) genes with the interleukin-1 receptor antagonist phenotype in the MI survivors (AIRGENE), Health 2000 study and FINRISK97 study populations. The direction of the association is described with increasing number of minor alleles. Underlined P-values remain significant after Bonferroni correction.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>MI Survivors</th>
<th>Health 2000</th>
<th>FINRISK97</th>
<th>Meta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SNP</td>
<td>Genotype</td>
<td>Beta</td>
<td>Effect</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL1A</td>
<td>rs3783521</td>
<td>38/185/159</td>
<td>-0.046</td>
<td>-12.66</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>rs1800587</td>
<td>37/168/181</td>
<td>-0.043</td>
<td>-11.88</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>rs2856838</td>
<td>45/163/160</td>
<td>0.084</td>
<td>23.46</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>rs3783548</td>
<td>0/68/313</td>
<td>-0.091</td>
<td>-23.33</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL1B</td>
<td>rs16944</td>
<td>56/162/169</td>
<td>0.006</td>
<td>2.03</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>rs3917356</td>
<td>78/158/133</td>
<td>0.009</td>
<td>4.24</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>rs1143634</td>
<td>24/148/213</td>
<td>-0.029</td>
<td>-0.11</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>rs1143642</td>
<td>1/37/349</td>
<td>-0.049</td>
<td>-12.62</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL1RN</td>
<td>rs2637988</td>
<td>96/192/99</td>
<td>0.012</td>
<td>3.15</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>rs3213448</td>
<td>15/131/241</td>
<td>0.052</td>
<td>34.89</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>rs315934</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs4252008</td>
<td>35/160/191</td>
<td>-0.025</td>
<td>-5.12</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>rs2232354</td>
<td>11/108/267</td>
<td>-0.052</td>
<td>-16.05</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>rs315952</td>
<td>46/176/147</td>
<td>0.124</td>
<td>40.55</td>
<td>4.2e-6</td>
</tr>
<tr>
<td></td>
<td>rs4252041</td>
<td>1/24/362</td>
<td>-0.281</td>
<td>-65.44</td>
<td>1.0e-4</td>
</tr>
<tr>
<td></td>
<td>rs315949</td>
<td>42/173/170</td>
<td>-0.107</td>
<td>-22.46</td>
<td>6.8e-5</td>
</tr>
</tbody>
</table>

1 Adjusted for age, gender, BMI, total cholesterol / HDL cholesterol -ratio, systolic blood pressure, current smoking, hypertension and residential area, 2 n (minor homozygotes / heterozygotes / major homozygotes), 3 effect per allele, 4 minor homozygotes and heterozygotes are pooled
5.3 IL-1RA, METABOLIC DYSREGULATION AND DEVELOPMENT OF DIABETES

5.3.1 Determinants of IL-1Ra phenotype

The proportion of the IL-1Ra phenotypic variation explained by IL1RN SNPs rs3213448, rs315952, and rs315949 ranged from 0.2% to 0.5% in the Health 2000 and FINRISK 97 populations and from 1.4% to 5.0% in MI survivors (original publication III). Rs315952 was found to be the strongest genetic predictor for the IL-1Ra phenotype in MI survivors and Health 2000 after adjustment for age and sex. The proportion of IL-1Ra variation explained by the IL1RN haplotypes (SNPs rs315952, rs4252041, and rs315949) was 0.7% and 4.0% in Health 2000 and MI survivors, respectively.

IL-1Ra levels increased with age and were higher in women than in men. The nongenetic traits predicting IL-1Ra are shown in Table 17. BMI and waist circumference were the strongest nongenetic predictors for the IL-1Ra phenotype. Body mass index explained 24.6%, 11.8%, and 18.1% of the IL-1Ra phenotypic variation in MI survivors, Health 2000, and FINRISK 97, respectively, when adjusted for age and sex. Waist circumference was an even slightly stronger predictor than body mass index explaining 13.6% and 20.3% of the phenotypic variation of IL-1Ra in two population samples. Glucose, insulin, triglycerides, and total cholesterol to HDL ratio levels were also significantly explaining the variation of IL-1Ra in the Health 2000 and FINRISK 97 populations. Systolic blood pressure was positively associated with IL-1Ra in the healthy population samples and the presence of hypertension or diabetes was associated with higher IL-1Ra levels in all three study populations.

5.3.2 IL-1Ra levels in metabolic syndrome and diabetes

The plasma concentrations of IL-1Ra and CRP differed clearly between participants free of metabolic syndrome (MetS) or diabetes at baseline, participants with MetS, and individuals with the presence of diabetes as can be seen in Table 18 (original publication IV). The pair-wise comparisons suggested that the main differences were between the “healthy” participants and the groups with dysregulated metabolism, whereas the differences between participants with MetS and diabetes were smaller.
Table 15. Insulin and glucose concentrations\(^1\) (geometric means) in the 2-hour oral glucose tolerance test (OGTT) in Health 2000 by copy number of the interleukin-1 beta (\(IL1B\)) haplotype ACG (SNPs rs1143634, rs3917356 and rs16944).

<table>
<thead>
<tr>
<th>Haplotypes (n)</th>
<th>0 min</th>
<th>(P^2)</th>
<th>(P)-trend</th>
<th>30 min</th>
<th>(P^2)</th>
<th>(P)-trend</th>
<th>120 min</th>
<th>(P^2)</th>
<th>(P)-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no copy (744)</td>
<td>55.4</td>
<td>-</td>
<td></td>
<td>298.7</td>
<td>-</td>
<td></td>
<td>269.5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>one copy (404)</td>
<td>57.2</td>
<td>0.14</td>
<td>0.021</td>
<td>304.2</td>
<td>0.66</td>
<td>0.78</td>
<td>274.2</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>two copies (46)</td>
<td>61.8</td>
<td>0.049</td>
<td>0.021</td>
<td>284.3</td>
<td>0.66</td>
<td>0.78</td>
<td>299.7</td>
<td>0.25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no copy (744)</td>
<td>5.70</td>
<td>-</td>
<td></td>
<td>8.63</td>
<td>-</td>
<td></td>
<td>6.45</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>one copy (404)</td>
<td>5.69</td>
<td>0.83</td>
<td>0.069</td>
<td>8.71</td>
<td>0.34</td>
<td>0.27</td>
<td>6.42</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>two copies (46)</td>
<td>5.99</td>
<td>0.004</td>
<td>0.69</td>
<td>9.24</td>
<td>0.008</td>
<td>0.27</td>
<td>7.60</td>
<td>0.0003</td>
<td>0.12</td>
</tr>
</tbody>
</table>

\(^1\)Adjusted for age, gender, BMI, waist circumference, mean systolic blood pressure, smoking, alcohol consumption, level of education and area of residence.

\(^2\)Subjects having no copies of tested haplotype were taken as the reference category and subjects having one copy or two copies of the tested haplotype were compared with them.

---

Table 16. The multivariate-adjusted associations with increasing copy number of haplotypes (SNPs rs315952, rs4252041 and rs315949) in \(IL1RN\) with the IL-1Ra phenotype in the MI survivors (AIRGENE) and Health 2000 study.

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>MI survivors</th>
<th>Health 2000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no/one/two</td>
<td>Beta</td>
</tr>
<tr>
<td>no copy</td>
<td>147/175/46</td>
<td>0.124</td>
</tr>
<tr>
<td>one copy</td>
<td>179/156/33</td>
<td>-0.071</td>
</tr>
<tr>
<td>two copies</td>
<td>178/157/33</td>
<td>-0.022</td>
</tr>
<tr>
<td>TTA</td>
<td>345/22/1</td>
<td>-0.276</td>
</tr>
</tbody>
</table>

\(^1\)Adjusted for age, gender, BMI, total cholesterol / HDL cholesterol -ratio, systolic blood pressure, current smoking, hypertension and residential area
Table 17. Non-genetic traits predicting the interleukin-1 receptor antagonist phenotype in the three study populations.

<table>
<thead>
<tr>
<th>Trait</th>
<th>MI Survivors</th>
<th></th>
<th></th>
<th>Health 2000</th>
<th></th>
<th></th>
<th>FINRISK 97</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta</td>
<td>Proportion (%)</td>
<td>P</td>
<td>Beta</td>
<td>Proportion (%)</td>
<td>P</td>
<td>Beta</td>
<td>Proportion (%)</td>
<td>P</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.005</td>
<td>1.1</td>
<td>0.038</td>
<td>0.003</td>
<td>0.6</td>
<td>7.9e-10</td>
<td>0.004</td>
<td>0.8</td>
<td>6.8e-14</td>
</tr>
<tr>
<td>Gender (reference: female)</td>
<td>-0.183</td>
<td>3.4</td>
<td>0.0003</td>
<td>-0.121</td>
<td>1.2</td>
<td>2.9e-18</td>
<td>-0.139</td>
<td>1.8</td>
<td>9.4e-29</td>
</tr>
<tr>
<td>Total cholesterol / HDL cholesterol ratio</td>
<td>0.098</td>
<td>6.1</td>
<td>4.2e-07</td>
<td>0.068</td>
<td>3.9</td>
<td>6.7e-57</td>
<td>0.107</td>
<td>7.3</td>
<td>3.4e-118</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>0.048</td>
<td>24.6</td>
<td>1.8e-26</td>
<td>0.041</td>
<td>11.8</td>
<td>3.0e-174</td>
<td>0.052</td>
<td>18.1</td>
<td>&lt;1.1e-201</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.001</td>
<td>0.3</td>
<td>0.27</td>
<td>0.002</td>
<td>0.4</td>
<td>2.2e-07</td>
<td>0.003</td>
<td>0.7</td>
<td>2.4e-12</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.080</td>
<td>0.9</td>
<td>0.053</td>
<td>0.168</td>
<td>1.3</td>
<td>3.3e-20</td>
<td>0.135</td>
<td>1.4</td>
<td>3.0e-23</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.110</td>
<td>0.1</td>
<td>0.64</td>
<td>0.091</td>
<td>0.4</td>
<td>1.1e-07</td>
<td>0.021</td>
<td>0.03</td>
<td>0.17</td>
</tr>
<tr>
<td>Prevalent diabetes</td>
<td>0.162</td>
<td>2.3</td>
<td>0.002</td>
<td>0.312</td>
<td>2.2</td>
<td>1.0e-32</td>
<td>0.194</td>
<td>0.7</td>
<td>3.1e-13</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td></td>
<td></td>
<td></td>
<td>0.017</td>
<td>13.6</td>
<td>&lt;1.1e-201</td>
<td>0.021</td>
<td>20.3</td>
<td>&lt;1.1e-201</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>0.001</td>
<td>0.7</td>
<td>3.4e-11</td>
<td>0.013</td>
<td>4.6</td>
<td>3.3e-72</td>
<td>0.024</td>
<td>0.3</td>
<td>9.7e-05</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>0.049</td>
<td>1.1</td>
<td>1.5e-17</td>
<td>0.024</td>
<td>0.3</td>
<td>9.7e-05</td>
<td>0.126</td>
<td>6.1</td>
<td>1.9e-99</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.114</td>
<td>4.4</td>
<td>5.4e-64</td>
<td>0.126</td>
<td>6.1</td>
<td>1.9e-99</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Age is adjusted for gender and gender is adjusted for age. All other variables are adjusted for age and gender,  proportion of variance explained by trait
Table 18. Baseline plasma Interleukin-1 receptor antagonist (IL-1Ra) and C-reactive protein (CRP) levels in men (M) and women (F) of the Health 2000 and FINRISK 97 cohorts. The participants are divided to three groups: persons with prevalent diabetes (DM) at baseline, persons with metabolic syndrome (MetS) according to the IDF criteria but no diabetes at baseline, and persons free of diabetes and metabolic syndrome at baseline (‘healthy’). Values shown are geometric means with interquartile range (IQR).

<table>
<thead>
<tr>
<th>Health 2000</th>
<th>Healthy</th>
<th>MetS</th>
<th>DM</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>1 403</td>
<td>1 034</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>IL1-Ra (µg/l)</td>
<td>267 (190 – 363)</td>
<td>342 (246 – 456)</td>
<td>367 (258 – 508)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>n</td>
<td>1 416</td>
<td>1 042</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.39 (0.20 – 1.40)</td>
<td>0.77 (0.41 – 2.22)</td>
<td>0.94 (0.41 – 3.20)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>1 859</td>
<td>964</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>IL1-Ra (µg/l)</td>
<td>305 (213 – 421)</td>
<td>401 (272 – 570)</td>
<td>531 (338 – 761)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>n</td>
<td>1 882</td>
<td>977</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.34 (0.16 – 1.29)</td>
<td>1.13 (0.56 – 3.31)</td>
<td>1.61 (0.80 – 4.61)</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FINRISK 97</th>
<th>Healthy</th>
<th>MetS</th>
<th>DM</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>2 499</td>
<td>972</td>
<td>286</td>
<td></td>
</tr>
<tr>
<td>IL1-Ra (µg/l)</td>
<td>207 (158 – 266)</td>
<td>284 (210 – 371)</td>
<td>269 (184 – 359)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>n</td>
<td>2 442</td>
<td>976</td>
<td>293</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.95 (0.44 – 1.85)</td>
<td>1.69 (0.81 – 3.02)</td>
<td>1.65 (0.64 – 4.07)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>2 892</td>
<td>666</td>
<td>253</td>
<td></td>
</tr>
<tr>
<td>IL1-Ra (µg/l)</td>
<td>239 (175 – 309)</td>
<td>359 (254 – 458)</td>
<td>327 (214 – 502)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>n</td>
<td>2 769</td>
<td>653</td>
<td>248</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.92 (0.41 – 1.88)</td>
<td>2.13 (1.11 – 4.07)</td>
<td>1.73 (0.65 – 3.95)</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

ANOVA is adjusted for age. Pair-wise post hoc analyses with Tukey’s tests: \(^1\) compared with group “Healthy” (P < 0.001), \(^2\) compared with group “MetS” (P < 0.001), \(^3\) compared with group “MetS” (P < 0.05)
5.3.3 IL-1Ra levels as predictors of incident diabetes

IL-1Ra level was a significant, independent predictor for the progression to clinically incident diabetes amongst men having metabolic syndrome in FINRISK 97 [HR (CI95%) per one unit increase in log(IL-1Ra), 1.70 (1.20 – 2.40), P = 0.003] and likewise in women [HR (CI95%) 1.70 (1.14 – 2.52), P = 0.008]. IL-1Ra was also a significant and independent predictor of progression to diabetes amongst men in Health 2000 [HR (CI95%) 1.81 (1.16 – 2.82), P = 0.009]. The risk for diabetes was also elevated amongst women in Health 2000 [HR (CI95%) 1.29 (0.82 – 2.03), P = 0.27], but did not reach statistical significance. When fasting glucose and CRP were added to the model, the hazard ratios for IL-1Ra attenuated substantially but remained significant with the exception of Health 2000 women (original publication IV).

Glucose level was the strongest predictor for the development of diabetes in Health 2000 men [HR (CI95%) per one unit increase in plasma glucose 3.49 (2.17 – 5.60), P = 2.4e-07] and women [HR (CI95%) 4.16 (2.57 – 6.74), P = 7.1e-09]. CRP, however, was significant only amongst women in the FINRISK 97 cohort (P = 0.005) and of borderline significance amongst men in Health 2000 (P = 0.049). CRP was a marginally stronger predictor for clinically incident diabetes amongst women than amongst men in FINRISK 97 (P = 0.049 for gender by CRP interaction), but the interaction did not reach statistical significance in Health 2000. IL-1Ra did not interact with gender in either cohort.

Metabolic syndrome (MetS) was observed at baseline in 2 090 (37.9 %) out of 5 511 participants in Health 2000 and in 1 740 (23.6%) out of 7 374 participants in FINRISK 97. During the follow-up of 7.1 years and 10.8 years, respectively, diabetes was found in 141 (6.8%) subjects with MetS at baseline in Health 2000 and in 248 (14.3%) subjects with MetS at baseline in FINRISK 97. Body-mass index was the most significant predictor for the progression to clinically incident diabetes amongst subjects with MetS in both study populations with hazard ratios (per one unit increase) ranging from 1.09 to 1.16 (original publication IV). Age was a significant risk factor for developing diabetes in FINRISK 97 and the hazard ratios were 1.03 per one year increase of age in both genders. A higher level of education was associated with decreased risk for incident diabetes in Health 2000 women. The presence of a cardiovascular disease event at baseline in Health 2000 men was associated with increased risk for developing diabetes.
6. DISCUSSION

6.1 SUMMARY OF THE MAIN FINDINGS

The results of this study suggest that genetic variations in IL-1, and in IL-1Ra in particular, play a role in glucose homeostasis and in the development of type 2 diabetes. Body mass index, waist circumference, other metabolic traits and genetic IL-1 variants are all significant contributors to IL-1Ra plasma levels. IL-1 family is also related to autoimmune responses.

The more specific findings include:

(1) The circulating IL-1Ra level is an independent predictor for the development of diabetes in people with metabolic syndrome, even after the adjustment for multiple factors including plasma glucose and CRP levels. The age-adjusted IL-1Ra concentrations are elevated in individuals with metabolic syndrome or diabetes when compared to those free of metabolic dysregulation.

(2) The levels of IL-1Ra are genetically determined by the IL1RN variants and some of the IL1B and IL1A variants when studied in a cross-sectional setting. However, the proportion of the phenotypic variation explained by the genetic factors is modest while body mass index or waist circumference, and other metabolic traits, are the major contributors for systemic IL-1Ra levels.

(3) Genetic variation at the IL-1 locus is broadly associated with glucose homeostasis traits at the population level. The coding IL1B SNP is associated with the traits related to insulin resistance.

(4) These results provide some evidence for a common IL1A promoter variation to act in predicting the development of type-2 diabetes among men, and suggested that the role may be gender specific. Likewise, common variation in the IL1B coding region may have a gender specific association for diabetes development.

(5) The systemic IL-1Ra levels may be elevated during a specific proinflammatory response and they correlate with CRP and ESR levels. Genetic variation in the IL-1 family seems to have an association with the appearance of TPO antibodies and with persisting local autoimmune responses and hypothyreosis following subacute thyroiditis.
6.2 METHODOLOGICAL ASPECTS

6.2.1 Study populations and design
These analyses included two large and representative Finnish population samples phenotyped in great detail. The cross-sectional analyses also included a multi-centre cohort of European MI survivors which was smaller than other population samples but included repeated measurements of inflammatory phenotypes. Additionally, OGTT was studied in a cross-sectional setting in a subsample of the Health 2000 cohort. The baseline data of Health 2000 and FINRISK 97 revealed some minor differences in age, gender and geographic distribution of the participants. Some differences in the baseline characteristics between the two population samples can be explained by the over representation of individuals over 80 years in the cross-sectional analyses of Health 2000. The sample of European MI survivors differed substantially from the population samples in several characteristics such as male predominance, older age and higher prevalence of diabetes. Lastly, the study of subacute thyroiditis was a relatively large disease orientated analysis of carefully defined consecutive patients and, later on, the follow-up examination data was available from the majority of participants initially studied.

This study is probably the largest reported prospective, population-based study of IL-1Ra phenotype as a predictor for incident diabetes including two separate observational cohorts with the same age range. A large number of individuals with metabolic syndrome were identified with a follow-up for the development of clinically incident diabetes. The ongoing medication was checked from the National Drug Reimbursement Register while the medications for hyperlipidemia in the cross-sectional analysis were elicited from the questionnaires. The follow-up period was longer for FINRISK 97 as compared to Health 2000 and the main outcome was identified from the national healthcare registers based on medications, hospital discharge diagnoses or cause of death diagnoses. Therefore, milder forms of diabetes and uncomplicated cases were not all included as cases in the prospective analyses. This may have weakened the observed associations slightly but is unlikely to have created spurious associations.

6.2.2 Genetic analyses
A large number of IL-1 variants were genotyped to cover variation in the IL-1 family of genes. The haplotype analysis of the IL-1 family was based on the definition of a
limited number of SNPs, each from one gene, to form a haplotype block (Barret et al. 2005, Gabriel et al. 2002). The patterns of LD and haplotypes in Health 2000 population and European MI survivors closely resembled each other. However, the imputation of the missing variants did not work well in our data ($r^2 < 0.30$), probably because the LD relationships in the Finnish population differ from those of the HapMap II CEU population (CEU, Utah residents with Northern and Western European ancestry from the Centre d’Etude du Polymorphisme Humain collection). The observed association of IL-1 variation with T2D or glucose homeostasis traits may reflect another causal variant, which is in strong LD with the variants determined in this study.

Study participants were of white European ancestry, and the findings cannot as such be generalized to represent other ethnicities; although, a recent genomewide linkage study on African-American families was consistent with our study reporting a strong candidate region for T2D in chromosome 2 (logarithmic odds–LOD score 4.53) extending from 41 to 121 Mb and including candidate genes in IL-1 and IL-1 receptor families (Elbein et al. 2009). A newly published genome-wide large-scale meta-analysis found no significant association at the genome-wide level for IL-1 variants with continuous diabetes-related traits in nondiabetic participants (Dupuis et al. 2010). The current study was based on a candidate gene approach and a genome-wide study would have provided a more comprehensive picture of the genetic background of the IL-1Ra phenotype. Nevertheless, a different genetic architecture for beta cell function and insulin resistance was suggested and only a few variants showed significant associations with insulin resistance as compared to several variants that associated with beta cell function. The associations observed in the Health 2000 data between one of the *IL1B* haplotypes and prevalent T2D were replicated in the European MI survivors and the power calculations showed that both materials were large enough to have an 80% power for detecting significant differences between the haplotype groups (zero vs. two copies) at the level of $\alpha = 0.05$. The prospective analysis for the genetic variants was small in our study and a larger study would be needed to provide a more comprehensive picture of the genetic effects IL-1 family.

The analysis of the rare variants with MAFs less than or equal to 0.1, enforced the pooling of rare allele carriers and thus the interpretation of the allele-dose relationships was not possible. Additionally, the interpretation of the results remained
inconclusive due to the relatively low frequencies of the rare variant SNPs despite some suggestive findings.

6.2.3 Glucose homeostasis traits

The most significant associations with glucose homeostasis were found for the coding rs1143634, which tags a common IL1B haplotype that also shows evidence of association. Plasma glucose and other related traits were analyzed in a cross-sectional setting in the Health 2000 population and therefore direct causal or environmental conclusions are not possible. The association of rs1143634 with plasma glucose was robust enough to survive the conservative Bonferroni correction for multiple testing. Plasma glucose was measured after fasting and 95% of the fasting periods were between 4 and 24 hours. Rs1143634 consistently showed suggestive associations for HbA1c and 2 hour glucose levels in OGTT, the latter of which is of substantial interest. The haplotype variation of three SNPs in the IL1B (rs1143634, rs3917356, and rs16944) associated with increased prevalence of diabetes, higher plasma glucose, OGTT glucose parameters, 2 hour insulin and HOMA-IR. It is therefore suggested that at least the two functional variants of IL1B, rs1143634 and rs16944, together may have a stronger association with insulin and glucose homeostasis traits than the single markers.

The association of IL-1 gene variation with glucose levels was more prominent than the association with insulin levels, although the two are metabolically closely related. The association with insulin levels available in the OGTT analyses may have been affected by the smaller sample size and younger age of the OGTT sample than the whole study population sample. Additionally, the association of prevalent diabetes was stronger among the MI survivors, who were older and predominantly males as compared to the larger random population sample. Two SNPs of the IL1A gene displayed a suggestive trend for an association with plasma glucose levels and one of them had a trend towards an association with HbA1c levels. These findings may reflect the significant LD between the variants of IL1B and IL1A. Furthermore, evidence was found to suggest an association between increased HbA1c and IL1RN rs4252041 which had the minor allele frequency of 0.02 denoting a relatively rare variant.
6.2.4 IL-1Ra phenotype

In this study, systemic IL-1Ra levels were determined by the corresponding gene or the gene family variation. The variation of three common IL1RN SNPs, rs3213448, rs315952, and rs315949, was associated with the IL-1Ra phenotype after correction for multiple testing and adjusting for multiple covariates. The findings were consistent in all study cohorts, with increasing significance levels in the meta-analysis, and survived the conservative Bonferroni correction. The homozygous genotype of the IL1RN rs3213448 minor allele was nominally associated with decreased insulin levels. The rare variant of the IL1B rs1143642 produced a clear statistical significance in the dominant model analysis and was associated with decreased IL-1Ra and with decreased 2 hour insulin levels. The associations of the IL-1 family gene variants were independent of body mass index and removing it among the covariates attenuated the genetic associations slightly, suggesting that they are not mediated by body mass index.

A trend for increasing IL-1Ra levels with increasing age was found in the healthy population samples. Additionally, female gender was clearly associated with increased IL-1Ra levels. The proportion of phenotypic variation in the IL-1Ra concentrations explained by the genetic variants was significant but modest in magnitude as compared with the proportion explained by body mass index. The haplotypic variation of three IL1RN SNPs (rs3213448, rs315952, and rs315949) was the strongest genetic predictor of the IL-1Ra phenotype. Waist circumference was the strongest nongenetic predictor of the IL-1Ra phenotype in two population based cohorts. The measured lipid parameters, total cholesterol to HDL cholesterol ratio, and triglycerides, consistently predicted circulating IL-1Ra concentrations that were most likely due to the underlying visceral obesity; the proportions of variances explained by these traits were lower than that seen with the body mass index. The presence of hypertension or diabetes was consistently associated with higher IL-1Ra levels. The proportions of variance explained by insulin and glucose were modest and comparable with the proportions explained by the genetic variants investigated. Furthermore, the nongenetic metabolic traits were associated with the levels of IL-1Ra or CRP and they were increased with prevalent diabetes or metabolic syndrome as compared to healthy individuals. During proinflammatory responses, such as subacute thyroiditis, the IL-1Ra levels correlated with CRP and ESR levels.
6.2.5 Outcome measures

A large number of individuals with metabolic syndrome were identified and they were followed for the development of clinically incident diabetes. The incidence of diabetes was somewhat higher in FINRISK 97 than in Health 2000 probably because of the longer follow-up time and larger waist circumferences. The main outcome was clinically incident diabetes, identified from the national healthcare registers, based on the use of hypoglycaemic medications, hospital discharge diagnoses, or death certificate diagnoses. Neither plasma glucose tests, nor OGTTs, were performed at baseline or during the follow up, thus individuals with diabetes treated with diet alone and never hospitalized were not identified and the same is true for people with undiagnosed diabetes. It is likely that this has attenuated the risk estimates somewhat.

The IL-1Ra levels were significant predictors for clinically incident diabetes in subjects with metabolic syndrome, after adjusting for a wide range of other risk factors, and remained significant after further adjustment for CRP and fasting glucose. The analyses amongst women showed that IL-1Ra was a significant predictor for diabetes in FINRISK 97. In Health 2000, with a smaller sample size, the direction of the risk was consistent but did not reach statistical significance. CRP was a somewhat weaker predictor than IL-1Ra. It was clearly significant in FINRISK 97 women and of borderline significance in Health 2000 men.

The predictive roles of the studied genetic variants were inconclusive, but together with earlier information suggest that variation in the IL-1 gene family may play a role in the pathogenesis of diabetes. In particular, the findings regarding \textit{IL1A} rs1800587 and \textit{IL1B} rs1143634 suggest that the associations between IL-1 gene variants and diabetes risk may be gender specific. Rs1800587 is associated with lower risk of clinically incident diabetes amongst women together with an interaction by gender. The genetic variants were nominally associated with incident diabetes and the associations did not survive the correction for multiple testing. Rs1800587 of \textit{IL1A} was associated with increased risk of diabetes amongst men, which is in agreement with the earlier findings of nominally higher blood glucose and IL-1Ra levels in Health 2000 cross-sectional analyses. Replication for the analyses of rs1800587 was not available. Rs1143634 of \textit{IL1B} was associated with increased risk of clinically incident diabetes amongst men in the prospective analyses of Health 2000. A nominally significant interaction by gender was observed in the pooled data. The same \textit{IL1B} variant was
consistently and robustly associated with increased glucose levels in Health 2000 and also with nominally increased HbA1c and IL-1Ra levels. The association for rs1143634 was not reproduced in the FINRISK 97 prospective analyses, and after pooling the cohorts, a weak protective effect was observed amongst women. The gender-specific effects may explain why the IL-1 locus has not been considered significant in the large meta-analyses of genome-wide association studies. The ACG haplotype of \textit{IL1B} was associated with prevalent diabetes, but in the prospective analyses no association with the development of diabetes was found. However, another \textit{IL1B} haplotype ATG was associated with an increased risk amongst men but not amongst women. Both these haplotypes are tagged by rs1143634.

Several of the SNPs that associated with IL-1Ra levels in the cross-sectional analyses were nominally associated with the risk of incident diabetes in at least one of the cohorts and at least one gender. Rs3213448 of \textit{IL1RN} was associated with increased IL-1Ra levels and approached significance for predicting diabetes in Health 2000 men but no association was found in FINRISK 97. Rs315949 of \textit{IL1RN} was associated with decreased IL-1Ra levels in the cross-sectional analysis and tended to be protective in both genders approaching statistical significance in men (Health 2000, dominant model).

Persistent thyroid autoimmunity was found in those patients who were clinically positive for thyroid antibodies at baseline investigation. Most of them were carrying IL1RN*2 of the VNTR polymorphism or the minor allele of \textit{IL1B} rs16944. Measurable levels of TPO antibodies were found only among women and persistent thyroid autoimmune responses were also observed in women only. However, the interpretation of a possible gender specific effect of the IL-1 variants on thyroid autoimmunity remains inconclusive because of the small size of the study cohort.

\section*{6.3 CURRENT FINDINGS AND OTHER STUDIES}

\subsection*{6.3.1 IL-1Ra levels and glucose homeostasis}

Prospective analyses of Whitehall II, FINRISK 97 and Health 2000 cohorts have previously shown that elevated IL-1Ra levels predict incident diabetes in middle-aged individuals (Herder et al. 2009, Salomaa et al. 2010). However, the hazard ratios were attenuated after adjusting for glucose levels and waist circumference. Metabolic syndrome is known to be a strong predictor for incident diabetes (Ford et al. 2008,
Pajunen et al. (2010). It was found in the present study that IL-1Ra is an independent predictor for incident diabetes in subjects with metabolic syndrome, at least in men, even after adjustment for glucose levels. The findings of the present study are also in agreement with the repeated measurements of IL-1Ra in the Whitehall II cohort that showed an accelerated increase in IL-1Ra concentrations during the 6 years prior to the onset of T2D. It is likely that the elevation of IL-1Ra is a compensatory phenomenon, reflecting the activity of the inflammatory process mainly driven by the proinflammatory IL-1β.

Previous experimental studies have shown that IL-1Ra is a natural inhibitor of IL-1β and improves beta-cell function and glycaemic control in patients with type 2 diabetes (Larsen et al. 2007, Larsen et al. 2009, Maedler (2) et al. 2004). A clinical study of long-acting anti-IL-1β antibody treatment in patients with type 2 diabetes aims to manage harmful consequences of glucotoxicity (Donath et al. 2008). Inflammatory factors are implicated in insulin resistance and beta-cell failure and inflammation precedes diabetes, while the levels of parameters denoting subclinical inflammation are comparable in subjects with impaired glucose tolerance and those with overt T2D (Kolb and Mandrup-Poulsen 2005). Elevated levels of inflammatory parameters such as CRP were predictors for T2D in people with different ethnic backgrounds and in different age groups (Kolb and Mandrup-Poulsen 2005, Pickup 2004). However, obesity partially attenuated the predictive capability, and in some studies, the association of inflammatory markers with type 2 diabetes was stronger in women than in men (Pickup 2004). Additionally, CRP was previously found as a stronger predictor for incident diabetes in women than in men (Thorand et al. 2007), a finding that was confirmed in our analysis of FINRISK 97. IL-1Ra remained a significant predictor for T2D after further adjustment for CRP and IL-1Ra concentration did not show any interaction with gender.

IL-1Ra levels were elevated in obese subjects with impaired glucose tolerance and metabolic syndrome when studied in cross-sectional design (Meier et al. 2002, Ruotsalainen et al. 2006). Previously, in patients with suspected coronary artery disease, the IL-1Ra levels were decreased in cases with T2D compared with nondiabetic individuals (Marculescu et al. 2002). Reasons for the difference with our current findings may be related to different patient characteristics. Our cross-sectional analysis confirmed, using two large population-based studies, higher levels of IL-1Ra at baseline
in individuals with T2D or metabolic syndrome compared with those free of metabolic
dysregulation. Similar findings were observed for CRP. Additionally, common oral
antidiabetic medications may partly explain the slight difference of IL-1Ra levels
between metabolic syndrome and T2D (Kolb and Mandrup-Poulsen 2005).

6.3.2 IL-1 gene variation and glucose homeostasis
Chronic exposure to increased IL-1β levels was followed by inhibited insulin signal
transduction and altered adipose tissue lipid content and differentiation (Lagathu et al.
2006). Extended exposure to IL-1β was capable of inducing insulin resistance by
decreasing insulin-induced glucose transport in adipocytes mainly by inhibiting insulin
receptor substrate-1 expression, the amount of which was dependent on the extracellular
receptor kinase pathway, and also a posttranscriptional mechanism independent of
extracellular receptor kinase (Jager et al. 2007). Short-term exposure to IL-1β was
followed by rapid glucose uptake by cells followed by counterregulatory responses of
increased glucagon, catecholamines, and glucocorticoids to maintain glucose
homeostasis (del Rey et al. 1998, del Rey et al. 2006). The present study shows
probably the first evidence for *IL1B* gene variants associating directly with blood
glucose and insulin resistance, suggesting impaired glucose uptake by cells further
predisposing to glucotoxicity.

Glucose-induced beta-cell production of IL-1β was shown to induce beta-
cell apoptosis and was suggested as a mecahnism in glucotoxicity (Maedler et al. 2002).
Human pancreatic beta-cells were capable of expressing IL-1Ra; but the expression was
decreased in patients with T2D and circulating systemic IL-1Ra levels did not
necessarily reflect the local situation in human pancreatic beta-cells (Maedler (2) et al.
2004). Rafiq and coworkers reported an association of the common variant *IL1RN*
rs4251961 with the IL-1Ra phenotype, together with a suggestive association for
metabolic traits (Rafic et al. 2007). However, a genome-wide meta-analysis was unable
to show any association between the *IL1RN* variants and T2D including the variant
being in strong LD with rs4251961 (Rafic et al. 2008). The present study found three
*IL1RN* variants that determined the IL-1Ra phenotype and that may play a role in
glucose homeostasis, including rs315949 that was associated with lower IL-1Ra levels
and is known to be in strong linkage disequilibrium ($r^2 = 0.84$) with rs4251961 which
had an increased carrier frequency in the group with a better response to anakinra in
treatment of type 2 diabetes (Larsen et al. 2009). It seems likely that some of the variants in our study indicate a disordered balance between the proinflammatory IL-1β and anti-inflammatory IL-1Ra proteins, which in turn leads to disturbed glucose and insulin metabolism.

Despite the strong linkage signal in chromosome 2, GWAS have not provided evidence for the association of IL-1 gene variants with T2D, whereas several other associated loci have been revealed (Elbein et al. 2009, Zeggini et al. 2008). The results are limited however, by the modest effect sizes of the individual common susceptibility variants and the need for stringent statistical thresholds that may cover some true associations or leave them in a grey area with insufficient statistical evidence. The findings presented here, on the association of \( \text{IL1B} \) with glucose homeostasis, were generally consistent with those of the Framingham study and with the Diabetes Genetics Initiative Scandinavian cohort (original publication II). The gender-specific effects may explain why this locus has not been detected as significant in large meta-analyses of genome-wide association studies.

Calpains are related to the synthesis of mature IL-1α (Sims and Smith 2010). The variation of the calpain-10 gene in chromosome 2 has been reported to have an influence on insulin sensitivity in nondiabetic subjects with only marginal evidence for increased risk of diabetes (Elbein et al. 2002). Additionally calpain-10 variation has been associated with T2D in the meta-analyses (Tsuchiya et al. 2006). A suggestive association between \( \text{IL1A} \) variation located in chromosome 2 and development of diabetes was found by prospective analysis in the present study, denoting a possible significant risk marker in men.

### 6.3.3 Metabolic traits and IL-1 gene variation affecting IL-1Ra phenotype

The expression and secretion of IL-1Ra from adipocytes were shown to associate with obesity (Juge-Aubry et al. 2003). The systemic levels of IL-1Ra were nearly 7-fold increased in morbidly obese patients compared with lean control subjects, and the insulin resistance index was the most important determinant of the IL-1Ra level (Meier et al. 2002). Additionally, a surgical intervention for obesity was followed by a significant decrease of the cytokine antagonist levels. Consistently with these findings the present study showed that waist circumference indicating visceral obesity and body-
mass index are the strongest nongenetic metabolic determinants of the IL-1Ra phenotype.

Association of the IL1RN variation with the IL-1Ra phenotype has been previously reported for some SNPs including a genome-wide analysis (Rafic et al. 2007, Melzer et al. 2008). A common IL1RN variant rs4251961 was associated with decreased IL-1Ra levels, in accordance with the IL1RN rs315949 in our study and both variants were tagging the same haplotype in Seattle SNPs ($r^2 > 0.65$). Rs4251961 was also associated with lower IL-1Ra expression after peptidoglycan stimulation of whole blood samples in 285 healthy persons recruited from the metropolitan Seattle area in the United States (Reiner et al. 2008). It has been suggested that a haplotype formed by rs2232354, rs315952, and rs315949 of IL1RN has an association with the IL-1Ra messenger RNA levels in another cohort of European MI survivors (van Minkelen et al. 2009). This is well in line with the present study, where rs315952 was associated with increased IL-1Ra level in MI survivors and in the healthy population sample.

A case-control study in patients younger than 50 years suggested an association of the IL1B rs16944 minor allele with atherothrombotic events and an association with IL-1β release from mononuclear cells (Iacoviello et al. 2005). It is also of interest that rs1143627 of IL1B, which is in strong LD with rs1143634 and in full LD with rs16944 (Chen et al. 2006) is associated with the transcription of IL-1β in the elderly Italian population (Melzer et al. 2008). In the present study rs16944 was not found to be a determinant for IL-1Ra phenotype and for rs1143634 the association with the IL-1Ra phenotype was only marginal in the healthy population sample. The presence of IL1RN*2 has been repeatedly associated with increased IL-1Ra levels from healthy individuals (Hurme and Santtila 1998, Strandberg et al. 2006 Reiner et al. 2008). However, a decreasing trend in IL-1Ra levels was found with SAT patients carrying IL1RN*2 while having an ongoing symptomatic proinflammatory response.

### 6.3.4 IL-1 gene variation and thyroid autoimmunity

The association of IL1RN VNTR variation with thyroid autoimmune disease has been suggested in Caucasian samples although a separate study was unable to show any association (Hunt et al. 2000, Heward et al. 2001). Additionally, the IL1B promoter variation was suggested to have associations with Graves’ disease or thyroid antibodies in study samples from Asian population (Liu et al. 2010, Hayashi et al. 2009). The
genetic results concerning the role of thyroid autoimmunity and IL-1 variation remain inconclusive. Some evidence for the association between thyroid autoimmune responses and IL-1 gene variation was found in the present study of the patients with SAT.

6.4 FUTURE PERSPECTIVES

6.4.1 IL-1Ra and metabolic dysregulation

Metabolic syndrome is a constellation of metabolic disturbances and therefore affected individuals with multiple risk factors need effective treatment to reduce the risk of diabetes and cardiovascular complications. Therefore it could be of clinical value to further distinguish high risk individuals with metabolic dysregulation as a target for intensive intervention. The present study suggests that IL-1Ra measurements may be useful in patients who could benefit from treatment interventions, but further research is needed to characterize the potential benefit in more detail.

The systemic blood levels of IL-1Ra do not completely represent the local situation at the cellular level. Additionally, medications may affect the inflammatory activation mediated by IL-1 family cytokines. Further studies in this area may improve our understanding of the biological pathways linking inflammation, obesity, and glucose and insulin metabolism.

6.4.2 Genetic architecture of IL-1 family and development of type 2 diabetes

The genetic results in the prospective analyses remained inconclusive, but together with the cross-sectional analyses they suggest gender-specific effects of the IL-1 variants on the risk of diabetes. Larger studies with more extensive genotyping are warranted including resequencing to further elucidate biological mechanisms for the observed associations. The difference between the IL1B rs1143634 genotype groups in plasma glucose levels was small, but the difference in the 2 hour plasma glucose levels in the OGTT was more substantial and may be of interest. However, the practical relevance of these findings remains to be seen. Pharmacogenetic studies on the effects of the IL-1 receptor blocking therapy may be indicated in the future with a possible clinical impact. Additionally, the role of IL-1 variation in atherosclerosis and atherothrombosis has not been thoroughly examined.
6.4.3 IL-1Ra phenotypic variation
The impact of genetic factors influencing IL-1Ra levels is incompletely understood. Further studies are warranted to reveal pathways linking IL-1 family or metabolic dysregulation. A genome-wide study would provide a more comprehensive picture of the genetic background of the IL-1Ra phenotype. Resequencing of specific regions in future studies may uncover variation of the quantitative trait of interest.

6.4.4 IL-1 family and adaptive immunity
Proinflammatory IL-1 activation is known to affect T cell polarization and further studies are needed to fully understand the development of adaptive immune responses. Additionally, novel extensive methods for measuring autoimmunity would be necessary. Larger studies with extensive genotyping may further elucidate the role of IL-1 gene variation in autoimmune diseases.

7. CONCLUDING REMARKS
The prospective analysis of two independent population based observational cohorts showed that IL-1Ra is an independent predictor for the development of diabetes and its predictive power was better than that of CRP in individuals with metabolic syndrome. The results of the present study confirmed that IL-1Ra concentrations are higher in individuals with metabolic syndrome or diabetes than in those free of these metabolic disorders. The IL-1 family proteins are known to play significant roles in glucose homeostasis and metabolic dysregulation. Recent literature has shown that IL-1Ra secreted from adipose tissue has beneficial effects on glucose homeostasis and a substantial clinical therapeutic and prognostic potential.

The results from our genetic analyses are broadly consistent with the previous studies of the IL-1 family, glucose homeostasis and metabolic dysregulation adding to the evidence that pro- and anti-inflammatory members of the IL-1 family play a role in the regulation of glucose homeostasis. A novel and consistent association of variation in the IL-1 family is suggested, especially for the IL1B rs1143634 with glucose homeostasis traits. Genetic variation in the IL-1 family is suggested to associate with persistent autoimmune responses. Three variants of the IL1RN gene and one of the IL1B gene were identified as independent determinants of the IL-1Ra phenotype in 2 or 3 separate populations. These associations were not mediated by obesity which was the
strongest nongenetic predictor of systemic IL-1Ra levels. The proportion of variance in IL-1Ra concentration explained by the IL-1 gene variation was statistically significant but modest in magnitude compared with the proportions explained by body mass index and other metabolic traits. The genetic analyses also suggest gender-specific effects of the $IL1A$ rs1800587 and $IL1B$ rs1143634 on diabetes risk.
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