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Genetic variation at the *IL1RAP* locus and its influence on late-life depression in a population-based sample in Gothenburg, Sweden.

Degree Project in Medicine

David Mårdensjö

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Supervisor: Anna Zettergren, Ph.D

Department of Psychiatry and Neurochemistry
Institute of Neuroscience and Physiology
Sahlgrenska Academy, University of Gothenburg

Abstract

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Author: David Mårdensjö
Department of Psychiatry and Neurochemistry at Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg
Gothenburg, Sweden, 2017

Introduction: Recently, it was discovered in two large genome-wide association studies that there is an association between genetic variation in the gene *interleukin-1 receptor associated protein* (*IL1RAP*) and Alzheimer's disease traits. Inflammation has been implicated as an important pathomechanism for both dementia and late-life depression (LLD). However, the association between *ILRAP* and LLD have to our knowledge never been specifically investigated

Aim: The aim of this study was to examine the possible association between LLD and genetic variation in, or in close vicinity, to the gene *IL1RAP* in a population-based sample of older individuals.

Methods: Genotype data were available for 3,559 study participants from four cohorts of the longitudinal gerontological and geriatric population studies in Gothenburg, and 2715 were included in the statistical analysis after exclusion. All participants took part in a neuropsychiatric and neuropsychological examination. The relation between genotype and depression diagnoses as well as with the severity of depressive symptoms (measured with MADRS-score) were investigated.

Results: The main findings were associations between the common homozygotes of *rs3773976*, *rs12053868*, *rs3773970* and *rs4687151*, and females with major depression, with the strongest being for *rs3773970* (OR: 2.01 [95% CI: 1.14-3.56], p=0.016) in the logistic regression model adjusted for APO ε4-status and age at first interview. Significant associations between the common homozygotes of two of these polymorphisms (*rs3773976* and *rs3773970*) and increased MADRS-score were also found in the linear regression model using the same covariates. No association was found for *rs9877502*.

Conclusions: Our results indicate that genetic variation at the *ILRAP* locus may be of importance for LLD. However, the effect size and study sample were small. The finding should be interpreted with caution until replicated in additional samples of older indviduals.

Key words: Late-life depression, gene, interleukin 1 receptor accessory protein

Contents

1 Introduction

1.1 Depression and late-life depression

Depression is a gruesome illness, first and foremost for the affected one but also for friends and relatives of the affected. The life time risk in the western world is expected to be between 9-19%, with women being more affected than men [1–3]. Measured in disability-adjusted-life-year (DALY), unipolar depression places itself at third place worldwide before diseases as ischemic heart disease and HIV/AIDS, according to the WHO[4]. Depression is a growing concern in the industrial part of the world, partly because it increases the all-cause mortality[5], and partly because of the huge economic burden it causes for society[6].

American Psychiatric Association has published the Diagnostic and Statistical Manual of Mental Disorders (DSM) that sets up criteria for the classification of mental illnesses and it is widely used internationally. Major depression is diagnosed based on nine criteria. At least one of the two core criteria (depressed mood and lack of interest) has to be present with at least four of the additional criteria (weight loss, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue, feelings of guilt or worthlessness, diminished ability to concentrate and recurrent thoughts about death or suicidal thoughts)[7]. Minor depression can be diagnosed when the patient exhibits at least two, but less than five, of the criteria[7]. The classification of depression is a controversial area, and other classifications have been proposed because of the heterogeneous clinical picture the disease exhibits[8].

It is argued that the clinical symptoms differ between depression with a first debut later in life and

early onset depression (EOD)[9]. Although there is no strict classification for late-life depression (LLD) it is usually said to be depression with a debut after 50-65 years of age. DSM-V does not take age of onset into consideration[7]. LLD is usually more associated with a cognitive and executive decline while EOD is associated with more emotional symptoms[9, 10]. Melancholic depression and psychomotor disturbances in depressed individuals seem to increase with age[11].

Major depression is quite uncommon in the older population (5%) even though many older people exhibits depressive symptoms (27%)[12]. Whether the incidence for depression change with age is still unclear[12]. Depressive symptoms in late-life are associated with psychological distress, decreased executive functions and lower life satisfaction[13]. LLD increases the all-cause mortality, especially among men[14]. In addition, suicide rates increase with old age[15], with depression being the strongest risk factor followed by other mental disorders and chronic somatic diseases[16]. Multi-morbid individuals, with diseases such as diabetes and cardiovascular insults, are also more likely to suffer from depression[17–19].

1.2 Genetic research

1.2.1 History and genetic research in general

Since its entrance, in the seventies, genetic research has given us tremendous amounts of new information and increased our understanding of disease and its causes. Monogenic disorders, like Huntington's disease, have been fully characterized and our understanding of them have increased tremendously[20]. Important knowledge has also been gained for multifactorial diseases, albeit with varying results.

Genetics can only in limited extent explain why certain individuals develop mental illnesses. A disease like schizophrenia is usually considered to have a strong genetic factor, compared to other

mental disorders, but in a famous Finnish adoption study with children born by mothers with schizophrenia it was shown that a healthy home environment is protective against the development of psychotic symptoms[21]. The same is true for depression where certain genes can predispose and set a lower threshold for developing depression - but it still requires that the individual is exposed to a certain number of negative life events[22].

Genetic polymorphism

The human genome is built up on roughly around three billion base pairs[23]. It is remarkably consistent over time and all humans share most of the genetic information. It is usually estimated that only about 0.5 % of the DNA vary between people[24]. The loci of variation are called alleles. Alleles occurring less than < 1 % in the population are regarded as mutations, while alleles occurring > 1 % are regarded as variants or polymorphisms[25].

Point mutations in the human genome can be caused by insertions, deletions and substitutions. A substitution occurring > 1 % in the population is also called a single-nucleotide polymorphism (SNP). SNPs are the most frequent variant in the human genome. SNPs tend to occur more frequently in non-coding regions, but they can still have an effect on the phenotype through transcriptional regulation and gene splicing. SNPs in coding regions can either be synonymous, leading to the same protein, or non-synonymous and coding for a different amino acid or a stop codon. Deletions or insertions in coding regions will change the reading frame and are often deleterious. Some SNPs affect human phenotype and predispose certain diseases. However, most have no known effect.[25, 26]

New SNPs are continuously discovered and up to date have millions been found. Each individual SNP gets a unique identifier, the so called “rsID”. Several databases have been constructed to keep track of all the SNPs. One of the most used one is the Single Nucleotide Polymorphism database (dbSNP: www.ncbi.hlm.nih.gov/SNP), where the records are updated every 1-2 months with new

SNPs.

There is also extensive structural variation in the genome which constitutes of inversions, translocations and copy number variants (CNVs). CNVs are variations in the number of copies of quite long DNA-stretches (>1 kb) caused by either duplications or deletions[25]. These structural variants affect human phenotype and in some extent cause human disease[25]. Unfortunately it has been hard to study structural variation in relation to human disease until recently[27]. With the increasing use and advances in microarray techniques and next-generation sequencing, it have been made possible to study CNVs further and new important findings have been discovered for some complex trait diseases such as autism[28]. Two additional polymorphisms are the repeats: short tandem repeats (STRs) and variable number of tandem repeats (VNTRs), where a short strand of DNA are repeated several times (2-13 nucleotide sequence for STRs and 10-100 nucleotide sequence for VNTRs)[29].

Linkage analysis and population based methods

Even though some genetic variants do not have any known effect, they can still serve as excellent markers for genetic research if they are located close to the region of interest. During meiosis and recombination, genes far away from each other tend to separate, while nearby regions are more likely to be inherited together - they are said to be in high linkage disequilibrium. This biological phenomenon lays the foundation for linkage studies and population-based studies.[26]

Linkage analysis dominated in the 1990s when much of the research was aimed at studying affected families with Mendelian disorders like cystic fibrosis and Huntington's disease[26]. Even though linkage analysis can be used for multifactorial diseases it is not preferred for studying complex trait diseases because it is hard to reach enough power[30]. Rather than using SNPs are the STRs more well suited for linkage analysis because of their high rate of variability and because they are multiallelic (in comparison with SNPs which are biallelic)[31]. This is an ad-

vantage when studying rare family clustered monogenic disorders[26]. About 300-400 STRs are required for conducting a linkage analysis[32].

Attempts to use linkage analysis when examining complex trait diseases, such as diabetes and mental disorders, have proved disappointing because of the small effect each gene contributes to the disease[30]. Finding an association between a genetic variant and a complex trait disease require larger samples which make population-based association studies a better choice[26]. Due to the STRs high variance[31], which risk to confound a large association study, will SNPs be more suitable because they are more stable over many generations and more plenteous than STRs[26].

Candidate gene association studies requires a prior hypothesis based on earlier research and findings, which is a limiting factor when studying complex trait diseases comprising of thousands of causative genes. Nevertheless, the candidate gene approach can be a decent tool to detect any effect when studying a rare allele or when having a rather small study sample. Markers are then chosen close to the candidate gene, which usually are SNPs. The SNPs can either be used as surrogate markers, i.e. they are in linkage disequilibrium with the region of interest, or they can be direct cause of the disease.[26, 33]

Linkage analysis and candidate gene association studies can in certain cases also be used in combination. Linkage analysis can first be used to find interesting regions in affected families and this information can then be extended to the whole affected population[26]. This approach was applied when APO $\varepsilon 4$ and its role in Alzheimer's disease (AD) was established[34, 35].

Genome-Wide Association studies (GWASs) have made it possible to scan the entire genome for risk factors for complex trait diseases without a prior hypothesis. With the completion of the HapMap project (HapMap: https://www.genome.gov/10001688/international-hapmap-project/), researchers had sufficient number of SNPs for conducting GWASs. Thousands of SNPs are required for conducting a GWAS. Because of multiple testing there is a high risk for false positives. This problem requires sufficient statistical correction for multiple testing. GWASs has provided

us with vast new information, but caution should be taken before making too big inferences since the enormous data that are given can be hard to interpret and put in a biological perspective. A GWAS with a positive association should be followed by fine mapping of the region and replication in an independent population. Consequently, GWASs can give us new clues and point us in the right direction, but further studies are required before we can make any inferences of the result.[36]

1.2.2 Neurobiology and genetics in relation to depression and late-life depression

It has been shown that depression tends to aggregate in families and that the heritability is expected to be up to 37-38 % [37, 38]. Many genetic risk factors have been found but they have been hard to replicate (even in the largest of GWASs), and their significance remains disputed[39]. The most likely explanation for this is that the studies have been under-powered[40]. A GWAS of depression usually requires tens of thousands participants[40]. Another possible explanation is that former studies have used a too heterogeneous study sample. Indeed, depression is a highly heterogeneous disease with different main symptoms, recurrence rates, age of debut, response to treatment etc.[41], hence could it be plausible to assume that the genetic background will differ between depressed individuals.

Monoamine hypothesis

Trying to solve the enigma of depression took off more than 60 years ago when the first effective antidepressants were discovered. With the discovery of Imipramine (tricyclic agent) and Iproniazid (monoamine oxidase inhibitor), and the surprising finding that they had beneficial effects in depressed individuals[42, 43], comprehensive research was initiated that would last several

decades in an attempt trying to understand the mechanisms behind these drugs, and through that understand the pathogenesis of depression. Today we know that these medications, as well as the newer antidepressants, work by immediately affecting the levels of serotonin, norepinephrine and in little extent dopamine. Famous hypothesis about these neurotransmitters were proposed during the 60s by Schildkraut (catecholeamine hypothesis)[44] and Coppen (serotonin hypothesis)[45].

Serotonin levels plays a vital role in certain depressed individuals, which have been shown through tryptophan-depletion studies and PET-studies[46, 47]. Several susceptibility genes have been found that codes for serotonin transporter (*SLC6A4)*, the serotonin receptors (*HTR1A* and *HTR2A*) and enzymes involved in degradation of monoamines (*MAO*) [48–52]. These genes and the gene for the norepinephrine transporter (*SLC6A2*) have also shown to affect the outcome of treatment with antidepressants[53–55]. The brain specific isoform of Tryptophan hydroxylase (*TPH2)* has also been implicated as a susceptibility gene[56], as well as genes involved in dopamine neurotransmission; dopamine D4 receptor (*DRD4*) and dopamine active transporter 1 (*DAT1/SLC6A4*)[57, 58].

However, it is a very simplistic view that depression should be caused only by a disturbance in the neurotransmitters: Firstly, even though the conventional antidepressants targeting the serotonergic neurotransmission change the levels of neurotransmitters immediately, they do not have a clinical effect until after up to 2-3 weeks[59], which indicates other mechanisms of action (i.e. changes in neuroplasticity and neurogenesis[60, 61]). Secondly, antidepressants can be used for several other conditions, distant from depression, like neuropathic pain and eating disorders[62]. Thirdly, not all individuals respond to therapy targeting the serotonergic neurotransmission, and there are several drugs not targeting the serotonergic neurotransmission for treating depression[63]. Nevertheless, serotonergic neurotransmission, and norepinephrine neurotransmission to some extent, keeps being a hot topic and generating articles. However, during recent years the scientific field has been shifting towards other pathways involved in the intricate pathogenesis of depression.

Hypothalamic–pituitary–adrenal (HPA) axis hypothesis

In the first quarter of the 20th century, it was discovered that depressed individuals have elevated levels of stress hormones[64]. Several published articles have shown that depressed individuals exhibits a hyperactive HPA-axis with elevated levels of Corticotropin-releasing hormone (CRH), cortisol and a reduced sensitivity for feedback inhibition[65–67]. It is believed that the hypercortisolemia seen in depression contributes to hippocampal atrophy, insulin resistance and weight gain in certain depressed individuals[68, 69]. Whether a deranged HPA-axis causes depression, or if it is just an epiphenomenal to depression itself, has been widely debated. However, studies have shown that people with trauma in their childhood, like sexual or physical abuse, are more likely to have a hyperactive HPA-axis, even if they are not currently depressed[70]. It has also been shown in rodents that maternal behavior affects DNA methylation pattern in glucocorticoid receptor and the ability to cope with stress in their offspring[71], and long-term antidepressant treatment seems to normalize the hyperactive HPA-axis[72]. Genetic variation in the glucocorticoid receptor also seems to affect treatment outcome with antidepressants[55].

Neurogenesis and neurotrophic factors

Reduced volume of the hippocampus and several structures in the prefrontal cortex are often seen in depressed individuals[68, 73, 74]. This could partly be explained by hypercortisolemia, as earlier mentioned, by inhibiting neurogenesis and causing neurotoxicity[75, 76]. However, other mechanisms of action have been proposed for this phenomenon, and brain-derived neurotrophic factor (BDNF) has gained a lot of interest. BDNF is important for neuronal integrity and neurogenesis[77–79]. Preclinical trials have shown that the BDNF concentration decreases in rodents exposed to chronic stress[80], and that antidepressant treatment can increase BNDF concentration[81]. BDNF directly infused into rodents hippocampus also have an antidepressant effect[82]. These results have also been replicated in human subjects through post-mortem

studies[83], as well as by studying living individuals by measuring serum BDNF[84]. However, the BDNF-hypothesis may need to be revalued because other studies have shown more or less the opposite effect of BDNF regarding depression[85, 86].

The *Val66Met* polymorphism in the *BDNF* gene, which has been implicated in many disorders, have been examined through candidate gene studies of depression but the results have been mixed and inconsistent[87, 88].

Inflammation and depression

Inflammation and its relation with depression has gained a lot of interest. Cytokines and other inflammatory proteins are often elevated during a depressive episode [89], and individuals administered Interferon, the main treatment for hepatitis C, suffer from mood alterations[90, 91].The inflammatory response affects both monoaminergic neurotransmission and the HPA-axis, two pathways implicated in the pathogenesis of depression[92, 93]. It is theorized that the interplay between depression and inflammation is a remnant from past times where stress or a potential threat would mount an inflammatory response in case of serious injury or infection. However, this response could be redundant and quite otiose in a modern society full of low threat stressors[94].

Anti-inflammatory drugs have shown promising results treating depression[95]. In addition, the common SSRI antidepressant also have an anti-inflammatory effect[96].

Genetic variations in the inflammatory mediators and their association with depression have been studied thoroughly, whereof a handful have been replicated in numerous studies, albeit sometimes with inconsistent results[97]. They include the cytokines *IL-1*β[98–100], *IL-6*[100, 101], *IL-10*[102, 103] and *TNF*-α[104–106]; the chemokine *MCP1*/*CCL2*[107]; the acute phase reactant *CRP*[108, 109] and Phospholipase A2 enzyme, *PLA2G4A*[110, 111].

Genes specifically implicated for late-life depression

LLD has not been studied in the same extent as EOD and the heritability is estimated to be lower[112], hence have the findings been modest. However, there is evidence that implicates that there could be an etiological difference between EOD and LLD[113]

Depressed older individuals often exhibit white matter lesions in the brain[114], hence has the term "vascular depression" been coined[115]. Much of the focus has been aimed at studying genetic variants in genes that could conduce vascular lesions. *APOE ε4* has long been a hot candidate in LLD but the results have been inconsistent[116–118].

5-10-methylenetetrahydrofolate reductase (MTHFR) is the rate determining step for the methylation cycle and thus crucial for DNA-structure and building amino acids. The methylation cycle requires folic acid and cobalamins as substrates. It is common practice to examine folic acid and cobalamins in depressed individuals, especially among the older population, since both folic acid and cobalamins are often decreased during depression and affect treatment outcome[119, 120]. Genetic variation in the *MTHFR* gene are also known for increasing the risk for vascular events through elevated homocystein levels[121], and an association was found between the *C677T* polymorphism and depression in one study[122]. However, a meta-analysis of five studies did not find any association[116].

Most of the candidate gene association studies have yielded inconsistent results. However, the largest meta-analysis of LLD up-to-date found modestly significant associations for *APOE*, *BDNF* and *SLC6A4*. These results differ slightly from meta-analysis conducted for EOD, which suggests different mechanisms of action.[116]

Interestingly, one of the largest GWASs of depression with 34,549 participants, with a mean age of 66.5 years, only found one significant association in the 5q21 region - a gene desert area[123]. This example illustrates clearly that genetic research for depression have methodological prob-

lems, where significant results from candidate gene associations studies and meta-analysis hardly never have been replicated in a GWAS. However, two genes have been found through GWASs and survived replication in further studies: *bicaudal C homologue gene 1* (*BICC1*) and *piccolo presynaptic cytomatrix protein* (*PCLO*)*[124]* Both genes are expressed widely in the brain and are important for synaptic connections and cell-to-cell communication[125, 126].

1.3 Depression and dementia - a shared pathogenesis?

It is well known clinically that depression and dementia are somehow intertwined and can be hard to distinguish from one another in the older population. Individuals suffering from depression, both in early- and late-life, are at an increased risk of developing dementia, and it seems that there is a dose-response effect in this relation where number of depressive episodes, duration and severity further increase the risk[127]. About 50 % of individuals suffering from LLD have a cognitive impairment[128], and even if the depression goes into remission many older individuals still have residual cognitive impairment[129]. On the other hand, individuals diagnosed with mild cognitive impairment (MCI) or dementia are more likely to develop depression[130, 131]. Depression could be an independent risk factor for developing dementia[127], but LLD could also be the first manifestations of a merging MCI and dementia, i.e. depression could be a prodromal symptom[127, 132].

The relationship, and the underlying mechanisms, between depression and MCI/dementia have generated many hypotheses. First there is the obvious one that individuals that start to develop cognitive difficulties get depressed just because of that. They may no longer be able to participate in social events and activities they once enjoyed and this cause withdrawal and apathy which could trigger or aggravate a depression.[133]

Another hypothesis comes from that depression goes with hypercortisolemia[65, 66], and reduced

levels of BDNF[84] which causes hippocampal atrophy[68]and structural changes in other parts of the brain as well[73, 74]. This could cause a mild but stable MCI in people with a low cognitive reserve that would wear off with antidepressant treatment, or it could cause earlier symptoms in individuals with a preclinical dementia that otherwise may have stayed undetected[134].

Cerebrovascular disease can cause, aggravate and maintain depression[114], hence the term "vascular depression"[115], and depression itself is a risk factor for cerebrovascular events[135]. Consequently, it is believed that this could be one of the mechanisms linking LLD and dementia (vascular dementia in particular[136], but cerebrovascular disease has also shown to contribute to AD[137]). Genetic research in evidence for this include, as aforementioned, genetic variation in *APOE ε4* which is associated with AD[138] and depression in some studies, albeit the results have been conflicting about the latter association[116–118]. Another gene that has been studied is the *ACE* gene which encodes the angiotensin converting enzyme. Genetic variation in the *ACE* gene have been associated with both depression and dementia[139].

Even though disturbances in the serotonergic neurotransmission mostly have been studied in relation to depression, there is some merging evidence linking serotonergic disturbances and AD. Healthy individuals treated with SSRI exhibit reduced levels of Aβ in the CSF, and AD mouse models treated with SSRI exhibits less Aβ in brain interstitial fluid and less senile plaque formation[140]. Chronic SSRI treatment has also shown to increase neurogenesis and protect cells against the cytotoxic effect of the Aβ-peptides[141]. One possible mechanism explaining this connection could be that there seems to be a relation between activity in the serotonin receptors and amyloid precursor protein metabolism[142, 143]. However, there is a lack of evidence for treating demented individuals with antidepressants solely for preventing disease progress in their dementia[144].

The most appealing hypothesis would be that certain types of LLD, MCI and dementia are all part of the same spectrum with the same underlying neurodegenerative mechanisms[132], but more neurobiological and genetic research have to be done before making such an assumption. How-

ever, there is some evidence that LLD share some of the neurodegenerative features that characterizes dementia. Individuals with a typical AD CSF-profile and increased uptake on a Pittsburgh Compound B Uptake Measurement (reflecting amyloid burden), in otherwise cognitive normal adults, are more likely to suffer from depression or develop depression and mood changes over time, suggesting that depression is prodromal symptom in AD for certain subtypes[145, 146].

As aforementioned, depression can cause a low grade inflammation in the periphery and central nervous system[89, 147]. Emerging evidence shows that is the case for AD as well. The focus has long been aimed at studying the senile plaques and neurofibrillary tangles which are the hallmarks of the disease[148]. Mutations in the genes encoding amyloid precursor protein and presenilin proteins have been found for familial forms of AD[149, 150], which gave the "amyloid hypothesis" further viability[151]. However, these results have been hard to extrapolate to the large population consisting of late-debut spontaneous AD. Instead has neuroinflammation been proposed as a crucial mechanism for AD development[152]. Indeed, individuals with AD exhibit very much the same neuroinflammatory picture as depressed individuals with increased neuroinflammation[153] and increased levels of cytokines in CSF and serum[154]. Cytokine levels are also increased further in individuals with AD that also suffer from depression[155]. In addition, genetic variation in inflammatory genes, *IL-1*β and *TNF*-α, have been associated with both AD[156, 157], and depression [98–100, 104–106].

It is now well established that both depression and AD involves neuroinflammation. Whether this is a secondary phenomenon or a pivotal mechanism is still unclear. However, multiple hypotheses about how neuroinflammation could contribute to respective disease, with a joint mechanism, have been proposed. Firstly, TNF-α correlates negatively with insulin-like growth factor 1 (IGF-1) in individuals with AD, suggesting that inflammation can lead to decreased levels of IGF-1[158]. IGF-1 is vital for neuronal integrity, normal brain development and for an efficient Aβ-clearence in the brain[159]. Lowered levels of IGF-1 are associated with AD[160] while higher levels probably are protective[161]. Similar correlations have been found for depression[162, 163]. Another

factor that decreases when subjected to neuroinflammation is the neurotrophic factor BDNF[164], which has been described earlier. Both depression [83, 84] and AD[165, 166] (and several other neurodegenerative diseases[167]) goes with decreased levels of BDNF. Lastly, a mechanism that is worth mentioning: Inflammation induce the enzyme indolamine-2,3-dioxygenase (IDO) and this enhances the metabolism of tryptophan to kynurenine. Tryptophan is the substrate for producing serotonin and hence could this enzyme induction cause serotonin depletion which could cause depressive symptoms[168]. In addition, the metabolite of kynurenine, 3-hydroxykynurenine, is neurotoxic and could contribute to the cognitive impairments seen in depression and AD[169, 170].

1.3.1 Specific background for conducting this study

In a recent longitudinal GWAS studying amyloid accumulation using F-florbetapir PET a strong association was found between the SNP *rs12053868*, located in the gene *Interleukin-1 receptor associated protein* (*IL1RAP*), and accelerated amyloid accumulation. This SNP was also associated with an increased cognitive decline, greater temporal cortex atrophy and decreased microglial activity. Deep sequencing of the IL1RAP gene revealed that six additional SNPs in the gene are associated with an increased amyloid burden. The authors suggest that microglial activity and the IL1/IL1RAP signaling pathway are vital for preventing amyloid accumulation and progression into AD.[171]

In another GWAS studying AD biomarkers in CSF the SNP *rs9877502* was found to be associated with increased CSF tau and p-tau levels as well as accelerated cognitive decline and risk for developing AD. The SNP *rs9877502* is located close to the non-coding RNA gene *SNAR-1*, but in close proximity lies several other genes widely expressed in the brain including *IL1RAP*. In a subsequent gene expression analysis it was also shown that *rs9877502* is associated with IL1RAP expression in the brain[172].

IL1RAP constitutes a vital part of the IL1-receptor which binds the cytokine IL1 which in turn take part in offsetting the inflammatory response during infection and trauma[173]. As earlier described, neuroinflammation has been implicated as a crucial mechanism in the pathogenesis of depression and AD. Genetic variation in the *IL1*β gene have been associated with both diseases[98–100, 157]. However, variation in the *IL1RAP* gene and the nearby SNP *rs9877502* have to our knowledge never been specifically studied in relation to depression. Since depression and dementia are believed to share certain risk factors and pathomechanisms, as previously described, it now seems plausible to examine the aforementioned SNPs in relation to LLD.

1.4 Aim

The aim of this study was to examine the possible association between five SNPs in, or in close vicinity, to the *IL1RAP* locus (*rs3773976*, *rs12053868*, *rs3773970, rs4687151* and *rs9877502*) and late-life depression in a population-based sample of older individuals, by investigating the relation between genotype and disease status as well as with the severity of depressive symptoms (measured using MADRS-score).

2 Methods and materials

2.1 Study population

The population of this study consists of 3559 participants from four different cohorts (PPSW-H70, H85, H95+ and H70 born 1944) of the longitudinal gerontological and geriatric population studies in Gothenburg. Participants have been collected from the Swedish population register based on birth dates.

Prospective Population Study of Women in Gothenburg (PPSW) and H70 born 1930

The PPSW is a prospective longitudinal and multidisciplinary study of women in Gothenburg born 1908, 1914, 1918, 1922 and 1930 on specific dates (6, 12, 18, 24 and 30). 1594 individuals were invited and 1462 agreed to participate (response rate = 91.7%) when the study commenced in 1968. The study has been described elsewhere[174]. Follow-up examinations have been done seven times since then, most recently in 2015.[175, 176]

The H70-study commenced in 1971 with participants born in 1901. The aim of the study was to study the normal aging process in both genders. New cohorts with 70-year olds have been added numerous times through the years. So far, there have been five cohorts whereof two have been studied longitudinally (1901-02 and 1930). The H70 cohort born in 1930, which is the one included in this study, has been described elsewhere[177, 178]. Participants born on specific dates

between January 1, 1930 and December 31, 1930 on specific dates (6, 12, 18, 24 and 30) and living in Gothenburg area were invited to participate. 1287 individuals were invited and 827 agreed to participate (response rate = 64%).[175, 176, 179]

PPSW and H70 born in 1930 were merged into one cohort in 2000. This cohort consists of women born in 1914, 1918, 1922 and 1930, and men born in 1930. Women born in 1908 were added to the H95+ cohort (see further below) because of low number of participants. Since 2000 have new participants been recruited to the merged PPSW-H70 cohort.[175, 176]

The number of participants from the PPSW-H70 cohort, where genotyping has been done, is shown in Table 1. Reasons for not being genotyped include: death, non-interest, relocated from Gothenburg or unable to get in contact.

Table 1: Participants from PPSW-H70

Year of birth	Women (*n*)	Men (*n*)	Total (*n*)
1914	43	0	43
1918	177	0	177
1922	223	0	223
1930	567	402	969
Total	1010	402	1412

H85

The H85 cohort is a mixed gender cohort of participants born in 1923-24 that commenced in 2009 with follow-ups in 2011 and 2013. The cohort has been described elsewhere[177]. Every second 85-year old born between July 1, 1923 and June 30, 1924 and living in Gothenburg area were invited to participate. 944 individuals were invited and 571 agreed to participate (response rate = 60.5%).[176]

The number of participants from the H85, where genotyping has been done, is shown in Table

2. Reasons for not being genotyped include: death, non-interest, relocated from Gothenburg or unable to get in contact.

Table 2: Participants from H85

Year of birth	Women (*n*)	Men (*n*)	Total (*n*)
1923	167	104	271
1924	151	95	246
Total	318	199	517

H95+

The H95+ cohort study started in 1996 and is probably the largest study in the world of mental disorders in people older than 95 years. The cohort has been described elsewhere[180]. In 1996-1998, all 95-year olds, born between July 1, 1901 and December 31, 1903 and living in Gothenburg area were invited to participate. 521 individuals were invited initially and 338 agreed to participate (response rate = 65%). Over time have more 95-year olds been recruited to the cohort.[176]

The number of participants from the H95+ cohort, where genotyping has been done, is shown in Table 3. Reasons for not being genotyped include: death, non-interest, relocated from Gothenburg or unable to get in contact.

Table 3: Participants from H95+

Year of birth	Women (*n*)	Men (*n*)	Total (*n*)
1901	2	0	2
1902	7	1	8
1903	23	6	29
1904	31	5	36
1905	37	6	43
1906	66	14	80
1907	71	17	88
1908	71	18	89
1909	59	13	72
1910	13	4	17
1911	8	3	11
Total	388	87	475

H70 born 1944

The H70 cohort consists of mixed-gender participants born in 1944 and the cohort was assembled in 2014. Every 70-year old born on specific birth dates (dates ending with 0, 2, 5 and 8) between January 1, and December 31, 1944 and living in Gothenburg area were invited to participate. 1666 individuals were invited and 1203 agreed to participate (response rate = 72.2%). So far, no data from this study has been published.[176]

The number of participants from the H70, where genotyping has been done, is shown in Table 4. Reasons for not being genotyped include: death, non-interest, relocated from Gothenburg or unable to get in contact.

Table 4: Participants from H70

Year of birth	Women (*n*)	Men (*n*)	Total (*n*)
1944	610	545	1155

2.2 Study procedures

Neuropsychiatric examinations and interviews

A clinical examination was conducted for each participant including psychiatric, somatic, functional and social tests at an outpatient clinic. If participants declined the examination they were instead offered an examination in the participant's home. A semi-structured close informant interview was also performed by telephone and included questions about intellectual function, psychiatric symptoms, activity level, dementia symptoms and changes over time. Also, regular follow-ups have been done for most of the participants (apart from H70 born in 1944, which will be followed up for the first time during 2019).

The psychiatric examination was either conducted by a psychiatrist, other medical doctor or a trained psychiatric research nurse. The medical doctors and psychiatric research nurses were trained by a psychiatrist and the inter-rater reliability for diagnosing dementia and depression was estimated to be between good and excellent (kappa-values ($\varkappa$) between 0.62-1.00 and 0.81-1.00 respectively).

The psychiatric examination and interview was semi-structured including Comprehensive Psychopathological Rating Scale (CPRS)[181], Montgomery-Åsberg Depression Rating Scale (MADRS)[182], Mini Mental State Examination (MMSE)[183] and rating of symptoms suggesting dementia which have been described previously[184].

CPRS is a 65-item rating scale measuring psychopathology and is used to asses various different psychiatric illnesses. It contains 40 self-reported items and 25 observed items. The scale can aid in setting diagnoses, follow changes over time and estimate severity of symptoms. Ever-day language is used in the scale so that it can be used by multiple professions in the psychiatric field

after little practice. [181]

MADRS is a sub-scale of CPRS and contains ten items for assessing depressive symptoms and evaluate response to treatment. Each item gives a value between 0-6 which in turn gives a total score ranging from 0-60. MADRS is often used clinically for classifying severity of depressive symptoms and follow response to treatment.[182]

Both CPRS and MADRS have been validated for older people[185, 186].

MMSE contains eleven questions that is used to swiftly, but roughly, assess the cognitive status of the participant. It is widely used clinically for the basal dementia investigation.[183]

Diagnoses

Major depression was diagnosed through DSM-IV by taking items from CPRS representing depressive symptoms. Major depression was diagnosed with the help of nine criteria: at least one of the core criteria had to be exhibited (depressed mood and/or markedly diminished interest or pleasure) and at least four of the additional symptoms (significant weight loss or weight gain or decrease or increase in appetite; insomnia or hypersomnia nearly every day; psychomotor agitation or retardation; fatigue or loss of energy; feelings of worthlessness or excessive or inappropriate guilt; diminished ability to think or concentrate, or indecisiveness and recurrent thoughts about death or suicidal ideation). The symptoms had to be present during the recent month. Dementia was not an exclusion criterion. Minor depression was diagnosed through DSM-IV-TR and required 2-4 of the aforementioned criteria. For this study, “any depression” was defined as having either major or minor depression.[187]

Dementia was diagnosed through DSM-IV[188] based on information and testing during the semi-structured interview. A close informant interview was conducted when possible. Informa-

tion about dementia status was also gathered from the Swedish Hospital Discharge Register.

2.3 Genetic markers and genotyping

DNA was extracted from blood samples and genotyped using KASPar PCR SNP genotyping system according to manufacturer's protocol (LGC Genomics, Hoddesdon, Herts, UK, http://www.lgcgroup.com/). The method utilizes two different forward primers, which enables one to distinguish heterozygotes from homozygotes, and a common reverse primer. Genotyping of *rs3773976*, *rs12053868*, *rs3773970, rs4687151* and *rs9877502* was conducted for this study. The SNPs were chosen based on that they all had been highly associated with AD disease traits in the two GWASs previously described[171, 172]. Several SNPs were found in the *IL1RAP* gene, but some of them showed high linkage disequilibrium ($r^2 > 0.8$) to the main finding in the study (*rs12053868*). Hence, the one's chosen for this study had a $r^2 < 0.8$[171]. All the genotyped SNPs were in Hardy-Weinberg equilibrium. None of the SNPs had a minor allele frequency (MAF) <1%. APOE genotyping was conducted as previously described[189].

2.4 Statistics

Genotype frequencies were compared between cases (i.e. participants that have ever suffered from a depression) and controls using chi-square test when sufficient number of participants in each group. Fischer's exact test was conducted under a dominant genetic model where the rare homozygote was grouped with heterozygote (i.e. TT and GT/GG for *rs3773976,* AA and GA/GG for *rs12053868*, CC and TC/TT for *rs3773970*, CC and GC/GG for *rs4687151* and GG and AG/AA for *rs9877502*) because there were so few carrying the rare homozygote in our study sample. Association between depression and the genotypes were analyzed using logistic regression analysis

in two steps under the same dominant genetic model. In the first step, age at first interview was added as a covariate. In the next step, both age at first interview and APOE ε4-status were added as covariates. All the above analyses were conducted separately for major depression, minor depression, and a third group "any depression" which means either major or minor depression. Participants were labeled as depressed if they ever had fulfilled the criteria for depression at any of the neuropsychiatric examinations and follow-ups. All the above analyses were in a subsequent step stratified for gender since we suspected that the effect could differ between the genders. We also conducted a moderation analysis with an interaction variable (sex×allele) to see whether the gender stratification was justified.

Association between the genotypes and MADRS-score were analyzed using linear regression in two steps using the same covariates. The analyses were first conducted for all participants and then only with depressed participants as well as stratified for gender.

All the statistical analyses were performed in SPSS 24 (IBM: www.ibm.com). Demented participants were excluded from all analyses. Consequently, 2715 participants were included in the statistical analysis (Figure 1).

Since this study is based on a pre-defined hypothesis we refrain from doing a correction for multiple testing. A Bonferroni correction would also be too conservative since our studies SNPs are not independent from each other but instead exhibit linkage disequilibrium to some extent. The α significance level was set to $p < 0.05$.

No power analysis was conducted since all the data had been gathered at an earlier time and we would not be able to affect the sample size. However, a candidate gene association study of depression with only 2715 participants is likely to be underpowered.

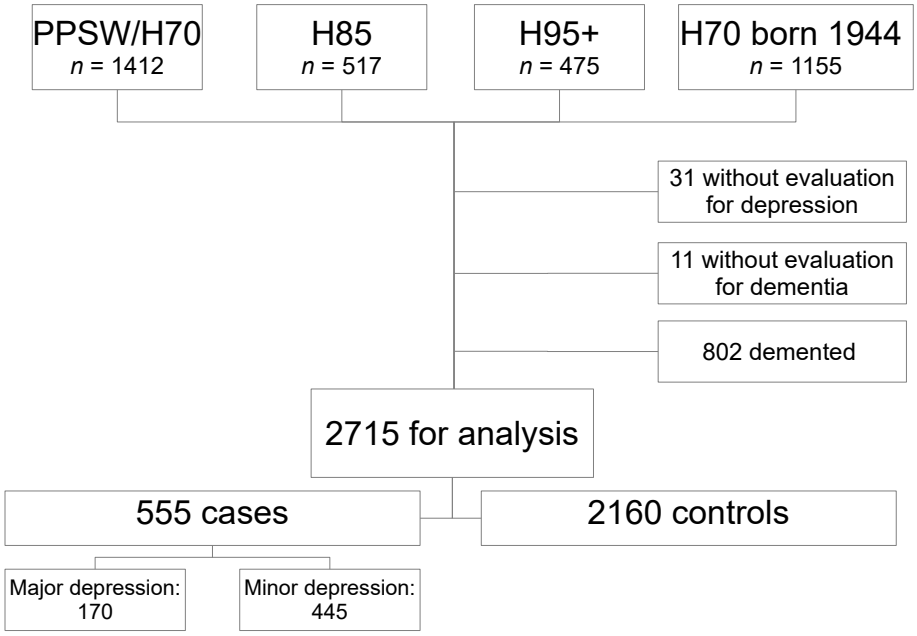

Figure 1: Flowchart of the study group

Genotype were available for 3,559 participants, albeit some of the participants had missing genotype data for a few of the SNPs due to genotyping errors. Participants without an evaluation for depression (31) or dementia (11) were excluded. All demented participants were excluded (802), leaving 2,715 participants included in the final analysis: 555 cases wherof 170 had ever fulfilled the criteria for major depression, 445 had ever fulfilled the criteria for minor depression and 2,160 non-depressed controls

2.5 Ethical consideration

The study has been approved by the Ethics Committee for Medical Research of the University of Gothenburg (diary number: s 069-01). Informed consent has been obtained from all participants and/or their relatives in case of dementia. All the data in the database were de-identified and each participant was instead given a unique serial number. Only a few members of the research team had access to identify individual participants if more information had to be gathered from the archive or journals.

3 Results

Characteristics of the study sample are presented in Table 5. Since the participants have been followed over many years, the same person can have been diagnosed with both major and minor depression at different follow-ups and hence appear in both groups, which causes the numbers to not add up.

Table 5: Characteristics of the Study Sample

	Ever any depression (*n* = 866)	Ever major depression (*n* = 255)	Ever minor depression (*n* = 696)	Never depression (*n* = 2662)	Total (*n* = 3528[c])
Gender (female, *n* and %)	661 (76.3)	202 (79.2)	532 (76.4)	1649 (61.9)	2310 (65.5)
Age at first interview (*m* and *SD)*	77.9 (10.4)	76.8 (9.4)	78.1 (10.7)	76.5 (10.1)	76.8 (10.2)
MADRS score (*m* and *SD)*	14.4 (7.6)	22.1 (7.4)	12.6 (6.4)	3.6 (3.5)	6.2 (6.7)
Demented *(n* and %)[a]	306 (35.5)	85 (33.3)	246 (35.6)	496 (18.7)	802 (22.8)
APO ε4 positive *(n* and %)[b]	249 (28.8)	63 (24.7)	208 (29.9)	738 (27.7)	987 (28.0)

[a] 11 without an evaluation for dementia
[b]3 without genotyping for *APO ε4*
[c]31 without an evaluation for depression and hence not included in this table

3.1 IL1RAP-related SNPs versus depression diagnoses

Associations were found for all the SNPs in the *IL1RAP*-gene (i.e. *rs3773976*, *rs12053868*, *rs3773970, rs4687151*) and females with major depression (Table 6, 7, 8 and 9). For all these findings the common homozygotes were the disease driving genotypes. All these associations were rather weak, with the strongest being for *rs3773970* (OR: 2.01 [95% CI: 1.14-3.56], p=0.016) in the logistic regression model adjusted for age at first interview and APOE ε4-status (Table 8).

We saw a trend towards significance in other groups: *rs12053868* in all participants with major depression (Table 7); *rs3773970* in all participants with major depression, as well as females with any depression (Table 8); *rs4687151* in all participants with major depression (Table 9). For these

trends the common homozygotes were the disease driving genotypes. Another trend was found between *rs3773976* and men with any depression where carriership of the minor allele appeared to be disease driving(Table 6).

No association was found for *rs9877502* (Table 10).

In the moderation analysis, we saw a trend towards significance for the interaction variable (p=0.052 when including the interaction variable in the logistic regression analysis for *rs3773970*).

Table 6: Association between *IL1RAP rs3773976* and depression status

Gene SNP	Any depression						Major depression						Minor depression					
	All		Female		Male		All		Female		Male		All		Female		Male	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
	N	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
IL1RAP rs3773976	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
TT	438	1691	329	993	109	698	139	1990	109	1213	30	777	347	1782	261	1061	86	721
	(80.2)	(79.5)	(83.1)	(79.8)	(72.7)	(79.0)	(83.7)	(79.4)	(87.9)	(80.0)	(71.4)	(78.4)	(78.9)	(79.8)	(80.8)	(80.6)	(73.5)	(78.7)
GT	105	406	65	236	40	170	26	485	14	287	12	198	90	421	60	241	30	180
	(19.2)	(19.1)	(16.4)	(19.0)	(26.7)	(19.3)	(15.7)	(19.3)	(11.3)	(18.9)	(28.6)	(20.0)	(20.5)	(18.9)	(18.6)	(18.3)	(25.6)	(19.7)
GG	3	30	2	15	1	15	1	32	1	16	0	16	3	30	2	15	1	15
	(0.5)	(1.4)	(0.5)	(1.2)	(0.7)	(1.7)	(0.6)	(1.3)	(0.8)	(1.1)	(0)	(1.6)	(0.7)	(1.3)	(0.6)	(1.1)	(0.9)	(1.6)
Statistical analysis																		
p^a	0.766		0.166		0.088		0.196		**0.033**		0.339		0.651		1.000		0.234	
OR (95% CI)[b]	1.05 (0.83-1.32)		1.24 (0.92-1.67)		1.43 (0.96-2.12)		1.34 (0.88-2.04)		1.82 (1.04-3.16)		1.46 (0.73-2.91)		1.06 (0.82-1.36)		1.02 (0.75-1.38)		1.34 (0.86-2.08)	
p^b	1.009		0.153		0.079		0.180		**0.035**		0.287		0.652		0.919		0.199	
OR (95% CI)[c]	1.04 (0.83-1.32)		1.24 (0.92-1.68)		1.43 (0.96-2.12)		1.34 (0.88-2.05)		1.83 (1.05-3.19)		1.46 (0.73-2.92)		1.06 (0.83-1.36)		1.02 (0.75-1.38)		1.34 (0.86-2.08)	
p^c	0.717		0.150		0.080		0.176		**0.033**		0.282		0.646		0.918		0.200	

[a] *p*-values comparing TT with GT+GG using Fisher's Exact Test

[b] Odds ratios with confidence intervals and p-values using logistic regression, comparing TT with GT+GG, with age at first interview as covariate

[c] Odds ratios with confidence intervals and p-values using logistic regression, comparing TT with GT+GG, with age at first interview and APO ε4-status as covariates

*42 participants without genotype for *rs3773976*

Table 7: Association between *IL1RAP rs12053868* and depression status

Gene SNP	Any depression						Major depression						Minor depression					
	All		Female		Male		All		Female		Male		All		Female		Male	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
	N	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
IL1RAP rs12053868	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
AA	443	1710	334	1012	109	698	146	2007	114	1232	32	775	347	1806	262	1084	85	722
	(81.9)	(80.6)	(84.8)	(81.8)	(74.1)	(79.0)	(86.4)	(80.5)	(89.8)	(81.9)	(76.2)	(78.4)	(80.5)	(81.0)	(82.4)	(82.6)	(75.2)	(76.6)
GA	96	387	58	212	38	175	23	460	13	257	10	203	82	401	54	216	28	185
	(17.7)	(18.2)	(14.7)	(17.1)	(25.9)	(19.8)	(13.6)	(18.5)	(10.2)	(17.1)	(23.8)	(20.5)	(19.0)	(18.0)	(17.0)	(16.5)	(24.8)	(20.2)
GG	2	24	2	13	0	11	0	26	0	15	0	11	2	24	2	13	0	11
	(0.4)	(1.1)	(0.5)	(1.1)	(0)	(1.2)	(0)	(1.0)	(0)	(1.0)	(0)	(1.1)	(0.5)	(1.1)	(0.6)	(1.0)	(0)	(1.2)
Statistical analysis																		
p^a	0.540		0.195		0.196		0.068		**0.028**		0.705		0.841		0.935		0.399	
OR (95% CI)^b	1.09 (0.86-1.39)		1.24 (0.91-1.69)		1.282 (0.86-1.93)		1.55 (0.98-2.43)		1.94 (1.08-3.49)		1.08 (0.52-2.24)		1.03 (0.79-1.33)		1.01 (0.73-1.40)		1.20 (0.76-1.89)	
p^b	0.490		0.178		0.230		0.058		**0.028**		0.835		0.839		0.941		0.444	
OR (95% CI)^c	1.09 (0.86-1.39)		1.24 (0.91-1.69)		1.28 (0.86-1.93)		1.55 (0.99-2.43)		1.95 (1.08-3.52)		1.09 (0.52-2.27)		1.03 (0.79-1.33)		1.01 (0.73-1.39)		1.19 (0.79-1.89)	
p^c	0.481		0.168		0.229		0.058		**0.026**		0.817		0.851		0.289		0.448	

[a] *p*-values comparing AA with GA+GG using Fisher's Exact Test

[b] Odds ratios with confidence intervals and p-values using logistic regression, comparing AA with GA+GG, with age at first interview as covariate

[c] Odds ratios with confidence intervals and p-values using logistic regression, comparing AA with GA+GG, with age at first interview and APO ε4-status as covariates

*53 participants without genotype for *rs12053868*

Table 8: Association between *IL1RAP rs3773970* and depression status

Gene SNP	Any depression						Major depression						Minor depression					
	All		Female		Male		All		Female		Male		All		Female		Male	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
	N	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
IL1RAP rs3773970	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
CC	436	1672	326	987	110	685	139	1969	109	1204	30	765	345	1763	258	1055	87	708
	(80.9)	(78.4)	(83.6)	(79.1)	(73.8)	(77.4)	(84.8)	(78.5)	(88.6)	(79.5)	(73.2)	(77.0)	(79.9)	(78.7)	(81.6)	(79.9)	(75.0)	(77.1)
TC	100	431	62	244	38	187	24	507	13	293	11	214	84	447	56	250	28	197
	(18.6)	(20.2)	(15.9)	(19.6)	(25.5)	(21.1)	(14.6)	(20.2)	(10.6)	(19.4)	(26.8)	(21.6)	(19.4)	(20.0)	(17.7)	(18.9)	(24.1)	(21.5)
TT	3	29	2	16	1	13	1	31	1	17	0	14	3	29	2	16	1	13
	(0.6)	(1.4)	(0.5)	(1.3)	(0.7)	(1.5)	(0.6)	(1.2)	(0.8)	(1.1)	(0)	(1.4)	(0.7)	(1.3)	(0.6)	(1.2)	(0.9)	(1.4)
Statistical analysis																		
p^a	0.215		0.058		0.345		0.061		**0.013**		0.572		0.652		0.530		0.640	
OR (95% CI)^b	1.17 (0.92-1.48)		1.34 (0.99-1.81)		1.22 (0.81-1.81)		1.52 (0.98-2.36)		2.00 (1.13-3.54)		1.22 (0.60-2.49)		1.07 (0.83-1.38)		1.12 (0.82-1.53)		1.12 (0.72-1.76)	
p^b	0.206		0.056		0.341		0.059		**0.017**		0.581		0.598		0.480		0.612	
OR (95% CI)^c	1.17 (0.92-1.48)		1.34 (1.00-1.82)		1.22 (0.81-1.82)		1.52 (0.98-2.36)		2.01 (1.14-3.56)		1.23 (0.60-2.51)		1.07 (0.83-1.38)		1.12 (0.82-1.54)		1.12 (0.72-1.76)	
p^c	0.209		0.054		0.339		0.060		**0.016**		0.566		0.606		0.481		0.617	

[a] *p*-values comparing CC with TC+TT using Fisher's Exact Test

[b] Odds ratios with confidence intervals and p-values using logistic regression, comparing CC with TC+TT, with age at first interview as covariate

[c] Odds ratios with confidence intervals and p-values using logistic regression, comparing CC with TC+TT, with age at first interview and APO ε4-status as covariates

*44 participants without genotype for *rs3773970*

Table 9: Association between *IL1RAP rs4687151* and depression status

Gene SNP	Any depression						Major depression						Minor depression					
	All		Female		Male		All		Female		Male		All		Female		Male	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
	N	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
IL1RAP rs4687151	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
CC	347	1304	257	771	90	533	114	1537	90	938	24	599	272	1379	200	828	72	551
	(63.3)	(60.8)	(64.6)	(61.9)	(60.0)	(59.4)	(68.3)	(60.9)	(72.0)	(61.8)	(57.1)	(59.6)	(62.0)	(61.2)	(62.1)	(62.6)	(61.5)	(59.2)
GC	175	745	123	424	52	321	45	875	30	517	15	358	147	773	108	439	39	334
	(31.9)	(34.8)	(30.9)	(34.0)	(34.7)	(35.8)	(26.9)	(34.7)	(24.0)	(34.0)	(35.7)	(35.6)	(33.5)	(34.3)	(33.5)	(33.2)	(33.3)	(35.9)
GG	26	94	18	51	8	43	8	112	5	64	3	48	20	100	14	55	6	45
	(4.7)	(4.4)	(4.5)	(4.1)	(5.3)	(4.8)	(4.8)	(4.4)	(4.0)	(4.2)	(7.1)	(4.8)	(4.6)	(4.4)	(4.3)	(4.2)	(5.1)	(4.8)
Statistical analysis																		
$\chi 2^a$	1.583		1.368		0.129		4.161		5.449		-		0.118		0.042		0.304	
p^a	0.453		0.505		0.937		0.125		0.066		-		0.943		0.979		0.859	
p^b	0.302		0.342		0.929		0.059		**0.027**		0.751		0.789		0.898		0.690	
OR (95% CI)[c]	1.12 (0.92-1.35)		1.12 (0.89-1.42)		1.04 (0.73-1.48)		1.39 (0.99-1.94)		1.59 (1.06-2.39)		1.09 (0.58-2.04)		1.03 (0.84-1.28)		1.02 (0.80-1.31)		1.11 (0.75-1.65)	
p^c	0.274		0.333		0.845		0.055		**0.024**		0.799		0.763		0.859		0.606	
OR (95% CI)[d]	1.11 (0.92-1.35)		1.12 (0.89-1.42)		1.04 (0.73-1.48)		1.39 (1.00-1.95)		1.61 (1.07-2.41)		1.1 (0.58-2.06)		1.03 (0.84-1.27)		1.02 (0.80-1.32)		1.11 (0.75-1.65)	
p^d	0.279		0.331		0.850		0.053		**0.022**		0.772		0.773		0.852		0.600	

[a] $\chi 2$-values and *p*-values after comparison between all genotypes using chi-square

[b] *p*-values comparing CC with GC+GG using Fisher's Exact Test

[c] Odds ratios with confidence intervals and p-values using logistic regression, comparing CC with GC+GG, with age at first interview as covariate

[d] Odds ratios with confidence intervals and p-values using logistic regression, comparing CC with GC+GG, with age at first interview and APO ε4-status as covariates

*24 participants without genotype for *rs4687151*

Table 10: Association between *rs9877502* and depression status

Gene SNP	Any depression						Major depression						Minor depression					
	All		Female		Male		All		Female		Male		All		Female		Male	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
	N	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
rs9877502	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
GG	206	799	151	464	55	335	60	945	47	568	13	377	169	836	123	492	46	344
	(38.6)	(37.8)	(39.2)	(37.7)	(37.2)	(37.9)	(37.0)	(38.0)	(38.5)	(38.0)	(32.5)	(38.0)	(39.3)	(37.7)	(39.3)	(37.8)	(39.3)	(37.6)
AG	262	1011	189	593	73	418	86	1187	62	720	24	467	207	1066	153	629	54	437
	(49.2)	(47.8)	(49.1)	(48.2)	(49.3)	(47.3)	(53.1)	(47.8)	(50.8)	(48.2)	(60.0)	(47.1)	(48.1)	(48.1)	(48.9)	(48.3)	(46.2)	(47.8)
AA	65	303	45	173	20	130	16	352	13	205	3	147	54	314	37	181	17	133
	(12.2)	(14.3)	(11.7)	(14.1)	(13.5)	(14.7)	(9.9)	(14.2)	(10.7)	(13.7)	(7.5)	(14.8)	(12.6)	(14.2)	(11.8)	(13.9)	(14.5)	(14.6)
Statistical analysis																		
$\chi 2^a$	1.638		1.447		0.253		2.945		0.945		3.072		0.908		0.977		0.137	
p^a	0.441		0.485		0.881		0.229		0.621		0.215		0.635		0.613		0.934	
p^b	0.727		0.631		0.927		0.867		0.923		0.511		0.551		0.650		0.762	
OR (95% CI)[c]	1.03 (0.85-1.26)		1.07 (0.84-1.35)		1.04 (0.73-1.50)		1.05 (0.75-1.46)		1.02 (0.70-1.49)		1.29 (0.66-2.55)		1.07 (0.86-1.32)		1.07 (0.83-1.37)		1.07 (0.72-1.59)	
p^c	0.738		0.597		0.822		0.780		0.919		0.459		0.544		0.617		0.747	
OR (95% CI)[d]	1.03 (0.85-1.25)		1.06 (0.84-1.35)		1.04 (0.73-1.50)		1.05 (0.75-1.45)		1.03 (0.70-1.50)		1.30 (0.66-2.56)		1.06 (0.86-1.31)		1.06 (0.82-1.37)		1.07 (0.72-1.59)	
p^d	0.764		0.613		0.824		0.790		0.885		0.449		0.573		0.649		0.738	

[a] $\chi 2$-values and *p*-values after comparison between all genotypes using chi-square

[b] *p*-values comparing GG with AG+AA using Fisher's Exact Test

[c] Odds ratios with confidence intervals and p-values using logistic regression, comparing GG with AG+AA, with age at first interview as covariate

[d] Odds ratios with confidence intervals and p-values using logistic regression, comparing GG with AG+AA, with age at first interview and APO ε4-status as covariates

*69 participants without genotype for *rs9877502*

3.2 IL1RAP-related SNPs versus MADRS-score

Significant associations between genotype and MADRS-score were found for *rs3773976* and *rs3773970* (Table 11). For *rs3773976* a significant association was found for women, both when including all participants and when including only depressed participants (p = 0.016 and p = 0.036 respectively). The common homozygotes were associated with a higher MADRS-score and hence more severe depressive symptoms. Among all participants with depression, both men and women, a significant association was found (p = 0.049), again with the common homozygote being associated with a higher MADRS-score. Lastly, a significant association was found for all male participants where carriership of the minor allele was associated with a higher MADRS-score (p = 0.041). For *rs3773970* significant findings were made for all participants and all females (p = 0.042 and p = 0.011 respectively), with the common homozygotes being associated with a higher MADRS-score. A trend towards significance was seen in females for *rs12053868* and *rs4687151.* No association was found for *rs9877502,* but for men with depression a trend towards significance was seen where carriership of the minor allele was associated with a higher MADRS-score (Table 11).

Table 11: Association between the SNPs and MADRS-score, demented excluded

	All subjects			Only depression (Any)		
	All	Female	Male	All	Female	Male
IL1RAP rs3773976						
MADRS (*M* and *SD)* TT	6.1 (6.6)	7.0 (7.1)	4.8 (5.5)	15.6 (7.5)	16.1 (7.7)	14.2 (6.7)
MADRS (*M* and *SD*) GT/GG	5.8 (5.9)	5.9 (6.0)	5.6 (5.7)	14.0 (6.3)	14.0 (6.3)	14.1 (6.2)
p-value[a]	0.262	**0.015**	**0.042**	**0.045**	**0.036**	0.920
p-value[b]	0.277	**0.016**	**0.041**	**0.049**	**0.036**	0.953
IL1RAP rs12053868						
MADRS (*M* and *SD*) AA	6.2 (6.6)	6.9 (7.1)	4.9 (5.5)	15.6 (7.5)	16.1 (7.7)	14.3 (6.7)
MADRS (*M* and *SD*) GA/GG	5.7 (6.0)	6.0 (6.1)	5.2 (5.8)	14.3 (6.4)	14.3 (6.5)	14.2 (6.4)
p-value[a]	0.116	0.054	0.569	0.097	0.098	0.966
p-value[b]	0.130	0.058	0.549	0.105	0.098	0.979
IL1RAP rs3773970						
MADRS (*M* and *SD*) CC	6.2 (6.6)	6.9 (7.1)	4.9 (5.5)	15.6 (7.5)	16.0 (7.7)	14.2 (6.8)
MADRS (*M* and *SD*) TC/TT	5.5 (5.7)	5.8 (5.8)	5.1 (5.5)	14.1 (6.2)	14.4 (6.2)	13.7 (6.3)
p-value[a]	**0.035**	**0.010**	0.579	0.072	0.115	0.749
p-value[b]	**0.042**	**0.011**	0.564	0.078	0.112	0.727
IL1RAP rs4687151						
MADRS (*M* and *SD*) CC	6.2 (6.7)	7.0 (7.2)	4.8 (5.4)	15.6 (7.5)	16.2 (7.7)	13.9 (6.5)
MADRS (*M* and *SD*) GC/GG	5.8 (6.2)	6.4 (6.4)	5.1 (5.7)	14.7 (6.9)	14.9 (7.0)	14.4 (6.8)
p-value[a]	0.197	0.083	0.486	0.185	0.098	0.702
p-value[b]	0.221	0.089	0.463	0.197	0.096	0.729
rs9877502						
MADRS (*M* and *SD*) GG	6.0 (6.4)	6.9 (6.8)	4.6 (5.3)	15.0 (7.2)	15.7 (7.3)	12.9 (6.6)
MADRS (*M* and *SD*) AG/AA	6.0 (6.5)	6.6 (7.0)	5.1 (5.6)	15.5 (7.3)	15.9 (7.6)	14.7 (6.6)
p-value[a]	0.922	0.409	0.119	0.372	0.830	0.063
p-value[b]	0.841	0.458	0.113	0.368	0.829	0.064

[a]Linear regression, comparing common homozygote with uncommon homozygote+heterozygote, with age at first interview as covariate

[b]Linear regression, comparing common homozygote with uncommon homozygote+heterozygote, with age at first interview and APO ε4-status as covariates

4 Discussion

4.1 Major findings

Previous GWASs have found an association between four SNPs (*rs3773976*, *rs12053868*, *rs3773970* and *rs4687151*) in the *IL1RAP* gene, and another SNP (*rs9877502*) in close vicinity to the *IL1RAP* locus and AD disease traits[171, 172]. Since depression and dementia partly share certain pathomechanisms, such as neurodegeneration, white matter lesions and genetic risk factors, we decided to conduct a candidate gene association study in a merged study sample of four of the longitudinal gerontological and geriatric population studies in Gothenburg applying a case-control study design. Our most prominent finding in this study was that the four SNPs located in the *IL1RAP* gene were associated with disease status for women with major depression, where the common homozygote appeared to be the disease driving genotype. Similar results seen for all these SNPs were not surprising since the four SNPs located in the *IL1RAP* gene (e.g. *rs3773976*, *rs12053868*, *rs3773970, rs4687151*) are in relatively high linkage disequilibrium with one another (r^2= 0.3-0.7)[171]. We saw a trend towards significance in other groups (i.e. men and women together and females with any depression), but it is obvious, by looking at the cross-tables, that these possible associations were driven by females with major depression. Significant associations between genotype and MADRS-score were found for *rs3773976* and *rs3773970,* and we saw a trend towards significance for *rs12053868* and *rs4687151*. Again, it seems, by looking at the table, that females were the disease driving factor for these associations and trends. A more surprising finding was for *rs3773976* (and in some sense *rs9877502* although not reaching significance) in men where carriership of the minor allele was associated with a higher MADRS-score.

However, we speculate that this could be a spurious finding since men with depression were few in comparison with women.

It is a known fact that depression involves neuroinflammation[147] (even though its role in the pathogenesis remains disputed) and elevated levels of several inflammatory substances in the periphery[89]. Genetic studies of depression have suggested several susceptibility genes related to inflammation, including *IL-1*β[98–100]. Our study suggests that genetic variation in *IL1RAP*, which constitutes a vital part of the IL1-receptor type 1, could possibly be of importance in the pathogenesis of LLD - or at least in older women with major depression. The physiological significance of ILRAP has been demonstrated in several animal experiments. As the name suggests, interleukin 1 receptor accessory protein, is just an accessory protein to the receptor. However, rather than mainly increasing affinity for IL-1, it is an essential part for signal-transducing to take place when IL-1 binds to its receptor[173]. During the inflammatory response, IL-1 and its binding to the IL1-receptor type 1 with a functional IL1RAP is crucial for activating the HPA-axis and increasing of IL-6 levels in the brain and periphery[190, 191]. If we assume that inflammation is a pivotal factor in depression pathogenesis, and that more inflammation correlates with more severe depressive symptoms, we can speculate that the major alleles in the four SNPs in the *IL1RAP*-locus renders in a more effective signal transmission when IL-1 binds its receptor and hence offsets a more powerful inflammatory response in these individuals (N.B. this is a very simplistic explanation since inflammatory pathways are known to interact with several other pathways important for depression pathogenesis[192]). This is in line with previous studies about depression and inflammation where certain groups of depressed individuals have increased levels of different inflammatory proteins, and that cytokines administered can induce a depressive episode in otherwise healthy adults[89, 90].

A study investigating these four SNPs in relation to dementia showed the opposite results, where the minor alleles were associated with AD traits, and the major allele could be seen as protective against AD[171]. Furthermore, the SNPs minor alleles were associated with both increased

amyloid burden and decreased microglial activity, suggesting that the minor alleles renders in a less powerful inflammatory response and decreased amyloid clearance[171]. Indeed, other studies have shown that IL-1 overexpression reduce amyloid burden while at the same time increasing tau-pathology in rodents[193]. Neuroinflammation has long been seen as pivotal mechanism in AD disease progression, but this assertion is now challenged since neuroinflammation seems to be beneficial when it comes to increase amyloid clearance and maybe even halt disease progression[171, 193]. However, this statement is not undisputed.

Although not the main aim of this study, we investigated possible associations between *IL1RAP*-related SNPs and dementia in our sample, but no association was found (see Appendix). However, our sample consisted of participants with different types of dementia and it is possible that these *IL1RAP*-related SNPs only are associated with AD.

There is a merging number of studies that present joint mechanisms in disease pathogenesis for depression and dementia. This study can conclude that it seems like the *IL1RAP*-locus is of possible importance for depression, but the disease driving genotype is then probably the one previously shown to be protective against AD. Earlier studies on partly the same population as in this study have also shown some distinct and surprising differences between depression and AD. Studies by Gudmundsson et. al. have shown that depressed females have a distinct CSF-profile with elevated levels of amyloid beta-42 (Aβ42), neurofilament protein light (NFL) and an increased CSF/serum albumin ratio but no changes in T-tau levels[194, 195]. This differ from the CSF-profile seen in AD with decreased levels of Aβ42 and elevated levels of T-tau and NFL[196]. As earlier mentioned in the introduction, most of the other studies examining CSF in individuals with LLD have shown that the CSF-profile is very similar to the AD CSF-profile[145, 146]. The contradictory results of studies examining CSF in depressed individuals probably reflect the heterogeneous nature of depression. Depression, and also LLD, is very much indeed a heterogeneous disease[8, 41], and certain subtypes of LLD may have a significant overlap with AD, while others do not.

In this study we only found an association between the four SNPs in *IL1RAP* gene and females with major depression. There are several possible reasons why we could not see any association among men: To start with, only 1053 men were included in the statistical analysis whereof 153 had been diagnosed with any depression and only 43 with major depression (after excluding individuals with dementia). Hence, there are limitations in statistical power and we cannot rule out that a possible association would be seen in a larger study group with more men with depression. However, genetics and heritability tend to differ between men and women when it comes to depression. Twin studies have shown that the heritability for depression is lower among men, 29 % for men and 42 % for women, and that genetic risk factors could be sex specific in their effect[38]. Thus, even though we lack statistical power, we could hypothesize that genetic variability in *IL1RAP* could be sex specific in its effect and hence of greater importance for predicting depression risk in women.

No significant association was found for minor depression, neither for women nor men, even though this group contained even more study participants. The most intuitive explanation for this is that minor depression is an even more heterogeneous disease than major depression. To acquire the diagnosis minor depression, in our study sample, participants have to fulfill one of the core criteria (depressed mood and/or markedly diminished interest or pleasure) and two of the additional criteria, which could be anything from "weight gain and increased appetite" to "psychomotor retardation and diminished ability to think". The diagnosis will be the same but the clinical picture could be very different. It is plausible to speculate that there are different pathomechanisms and genetic risk factors involved in these vastly different disease presentations. In addition, among older people, a chronic mental disorder often underlies a major depressive episode, which is linked to a more vulnerable personality and a greater familial heredity, while minor depression often tend to be a reaction to life stressors (i.e. illnesses, death of spouse, loneliness etc.)[197]. However, some people argue that major and minor depression are in a single continuum with the same genetic risk factors, and the heritability for minor depression have been expected to be 37%

in one study[198]. Under these conditions, an association should have been found for minor depression, or at least for the total group "any depression", and our main finding in depressed females could be dismissed as a spurious finding.

4.2 Strengths and weaknesses

A strength of this study is the population-based design, which make our study sample representative of the whole population. In addition, the study group were very homogeneous, consisting mostly of white northern Europeans which increases the chances of finding a possible association. Another strength was the thorough examination of each participant, by either a psychiatrist or a trained psychiatric nurse, and that there have been several follow-ups for most of the participants with high response rates. This is very important for being able to detect all participants with a predisposition for LLD since depression often goes with remissions and relapses.

This study had several limitations. Firstly, our sample size of 2715 participants in the statistical analysis is rather small which is reflected in our significant findings that would not survive a correction for multiple testing. This does not rule out that there is an association between *IL1RAP* and LLD, but it is still possible that this could be a spurious finding. Another weakness is that women were over-represented in this study, since the PPSW cohort consisted solely of women. There were too few men in this study, and a possible association could have been missed. Lastly, it is difficult to differentiate between depression with a debut later in life and recurrent depression in late life, which is a confounding factor when conducting studies on LLD, and much of the depression seen in the older population is partly caused by chronic pain, neurological disorders, poly-pharmacy and various different somatic illnesses which we did not take into account in this study.

4.3 Future directions

First off, future studies will require much larger study samples since the effect sizes are really small when it comes to depression. To find and establish a solid association with some certainty requires tens of thousands of participants. The whole research field struggles with this issue since it is extremely costly and demanding to include that many participants. One way to possibly by-pass this obstacle would be to purify the group of participants with depression. Because of the heterogeneous nature of depression it is highly likely that the genetic risk factors will be different. One approach would be to only include depressed with similar main symptoms and with no other comorbidities, and maybe also only include women since the heritability is much higher. Then the chance will increase that they share similar genetic risk factors. This is of course a tradeoff, we would increase our power, but we will miss out on most of depressed individuals.

Another possibility would be to distinguish LLD from recurrent depression in late-life, since there are some striking differences between LLD and EOD, probably with different risk factors[9]. When studying genes that have been implicated in Alzheimer's disease it is presumably better to have a study sample consisting solely of depression with a first debut later in life. However, we did not have any reliable information about depressive episodes at younger age in our study sample. Most people recall earlier episodes of sadness during life and it is hard for the assessor to determine if this represents true depression or just normal rational sadness.

Lastly, it would be interesting to follow all the study participants longitudinally and to see who eventually develops dementia and who do not. As earlier mentioned there could be subtypes of LLD that lies closer to dementia than others. We speculate that the findings for *IL1RAP* could be different between these two subgroups.

4.4 Conclusions

In this population-based cohort we found modestly significant associations between four SNPs in the *IL1RAP* gene and females with major depression as well as with MADRS-score for two of the SNPs. The effect size and study sample were small and replication in larger study cohort is required to strengthen this association. However, there is now some evidence that genetic variation in the *IL1RAP* gene, which have been implicated in AD, could be of importance in LLD as well. Both LLD and AD are in desperate need for new treatments and alternative pathways like IL1/IL1RAP may prove to be of importance in the future.

5 Populärvetenskaplig sammanfattning

Genetisk variation i genen IL1RAP och dess koppling till depression hos äldre

Depression kan drabba människor i alla åldrar. Vissa drabbas bara en gång medan det för andra kan återkomma genom hela livet. En särskilt utsatt grupp i samhället är äldre människor som ofta lider av depressiva symptom. Ofta förklaras det med att man som gammal drabbas av sjukdomar och ensamhet. En meningsfull tillvaro och en god fysisk hälsa är i allra högsta grad viktigt för det psykiska välbefinnandet - men det kan inte ensamt förklara varför vissa drabbas av depression.

Forskning har visat att en del av svaren till varför depression uppstår går att hitta i våra gener. Även om vi människor delar 99,5% av vårt genetiska material finns det små subtila skillnader i den genetiska koden som gör varje människa unik. Dessa skillnader påverkar hur vi ser ut men också vår risk att insjukna i olika sjukdomar. När det gäller depression påverkar varje enskild genetisk variation ganska lite men sammantaget kan flera av dessa variationer sänka vår tröskel för att insjukna i depression. Man har tidigare hittat kopplingar mellan depression och variation i gener som kodar för signalsubstanser, tillväxtfaktorer, inflammationssubstanser mm.

Hos äldre människor kan det ibland vara svårt att särskilja på demens och depression. I viss mån delar sjukdomarna symptom såsom koncentrationssvårigheter, förlorat intresse för aktiviteter och intresseområden samt social isolering. Det finns också belägg för att sjukdomarna delar vissa sjukdomsmekanismer och genetiska riskfaktorer. Inflammatoriska processer i hjärnan har visat sig ha betydelse för både depression och demens. Nyligen har forskare hittat belägg för att genetisk variation i genen som kodar för interleukin 1 receptor accessory protein (IL1RAP) kan påverka risken för att utveckla Alzheimers sjukdom. Denna gen är viktig för inflammatoriska signaleringsvägar. Denna gen har aldrig undersökts i relation till depression hos äldre. Därför har vi i denna studie undersökt denna gen hos 2715 studiedeltagare som ska representera Göteborgs äldre befolkning.

Vi fann ett samband mellan genetisk variation i *IL1RAP* och kvinnor med svår depression. Vi hittade inget samband för män och för personer med lättare depression. Våra resultat var ganska svaga och studien skulle behöva upprepas i en studie med fler deltagare, där också fler män inkluderas, för att kunna påvisa ett tydligare samband. Trots detta finns det nu ett visst belägg för att *IL1RAP* skulle kunna vara viktig när det gäller risken att utveckla svår depression hos äldre kvinnor. Genetisk forskning av depression är viktigt för att förstå sjukdomsmekanismer ytterligare och kan på sikt öppna för nya behandlingsmetoder.

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A Appendix

Table 12: Association between the SNPs and dementia status

	Dementia	No Dementia	Chi-square		Logistic regression[a]	
Gene: SNP	*N* (%)	*N* (%)	$\chi 2$	p-value	OR	p-value
IL1RAP: *rs3773976*						
TT	629 (80.3)	2129 (79.6)				
GT	149 (19.0)	511 (19.1)	1.994	0.369	1.082	0.511
GG	5 (0.6)	33 (1.2)				
IL1RAP rs12053868						
AA	635 (80.1)	2153 (80.9)				
GA	152 (19.2)	483 (18.1)	0.717	0.699	1.008	0.948
GG	6 (0.8)	26 (1.0)				
IL1RAP rs3773970						
CC	609 (77.4)	2108 (78.9)				
TC	172 (21.9)	531 (19.9)	2.400	0.301	1.066	0.574
TT	6 (0.8)	32 (1.2)				
IL1RAP rs4687151						
CC	496 (62.3)	1651 (61.4)				
GC	271 (34.0)	920 (34.2)	1.053	0.591	1.084	0.406
GG	29 (3.6)	120 (4.5)				
rs9877502						
GG	298 (38.6)	1005 (38.0)				
AG	364 (47.2)	1273 (48.1)	0.225	0.894	1.037	0.711
AA	110 (14.2)	368 (13.9)				

[a]Logistic regression comparing common homozygote with uncommon homozygote + heterozygote, with age at first interview, APO ε4-status and gender as covariates.