Identification and Functional Characterization of Genetic Risk Factors in Alzheimer´s Disease

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SUMMARY

Alzheimer's disease (AD) is the most common neurodegenerative disease in industrial nations. Its prevalence increases with advanced age. The most important histopathological markers in the brain of AD-patients are neuritic Aβ plaques and neurofibrillary tangles, contributing to the degeneration of neurons in the brains of affected patients. Numerous epidemiological studies suggest that genetic factors - together with lifestyle and environment dependent determinants – have an implication on the development of this multifactorial disease. Mutations in three genes (*PS1*, *PS2* und *APP*) cause an autosomal dominant, early-onset familial form of AD (EOAD). A fourth gene, *ApoE*, increases the risk of the more common late-onset familial (LOAD) and sporadic forms of AD, when the *ApoE* ε4 allele is present. Moreover, several linkage studies have defined susceptibility regions for AD on several chromosomes.

In the present work, a fine-mapping of functional and positional candidate genes was carried out in previously identified linkage regions. Selected SNPs were genotyped in a local case-control collective and were subsequently tested for an association with Alzheimer's disease. The focus of the presented work was the *APP* gene and genes involved in the processing of APP and therefore Aβ formation.

39 SNPs from *APP* were thoroughly selected and subsequently genotyped by means of Matrix-Assisted Laser Desorption / Ionization Time Of Flight (MALDI TOF MS). The initial statistical single-marker, haplotype and sliding-window analyses revealed significant association signals ($p < 0.05$) within two marker peak regions spanning intron 3 to 8 and in intron 1, respectively. A selection of SNPs with strongest association signals underwent replication in a second large independent case-control series from Sweden, including a pooled analysis, as well as a discordant sib-pairs cohort. The replication analyses confirmed signals for eight SNPs (rs9941877, rs2830012, rs3787644, rs2070655, rs2830035, rs1041420, rs6516727 und rs2830099) and therefore, their implication to Alzheimer's disease. A following association analysis of genotyping results from all 39 SNPs with clinical measures (MMSE score and age at onset) yielded a positive result for rs1783016 in intron 14 of *APP*. The risk allele (rs1783016-G) appears to be coupled with a later age of onset, but a more rapid decline in cognition. Subsequently, SNPs from the peak regions underwent further analyses in post mortem isolated brain samples to ascertain the functional implications of polymorphisms across the *APP* gene on the transcriptional, translational and post-translational level. The identified risk allele (T) of SNP rs1041420 located in intron 3 was associated with elevated APP mRNA levels in the brain, suggesting that the rs1041420-T allele might contribute to the

development of AD due to altered amounts of APP protein and not due to altered APP processing. For SNPs rs6516727 and rs2830099 - located in intron 1 - an association of the formerly identified risk allele (rs6516727-T and rs2830099-G) with lower soluble total Aβ and lower Aβ40 contents was found. The respective protective C variants (rs6516727-C und rs2830099-C) seem to favor the formation of the soluble Aβ40. The majority of present Aβ42 may already have formed plaques in the affected brains. Therefore, the risk alleles (rs6516727-T and rs2830099-G) are rather associated with altered processing than higher substrate levels, since no affect on the gene expression was observed. The applied approach - examination of functional implications of the identified SNP variants in brain samples - was well-chosen and is applicable for our experiments, but needs to be repeated in a greater collective. From the data analysis performed in the scope of this study, it is assumed that common variations within the *APP* gene contribute to the development of AD.

The proteins encoded by *ADAM10* and *BACE1* are involved in APP processing and therefore the development of Aβ, due to their function as α- or ß-secretase, respectively. Hence, 38 SNPs from both genes were thoroughly selected, genotyped and statistically evaluated accordingly. None of the examined SNPs from *ADAM10* or *BACE1* showed a significant association with AD. For this reason it is assumed that common genetic variations in either *BACE1* or *ADAM10* are not associated with an increased risk to develop AD.

Furthermore, two genes (*ABCA1* and *LIPC*) involved in lipid metabolism were examined. Two of the 30 investigated SNPs from *ABCA1* showed a weak association with AD (rs2525606 and rs2066720). The results could be confirmed in the Swedish replication study. During the following gene expression experiment the risk allele rs206676-G was associated with elevated ABCA1 mRNA levels in brain samples, probably due to increased ApoE lipidation. The 25 investigated SNPs from *LIPC* showed no replicable significant association with AD, therefore seems an impact of SNP variants in *LIPC* on the implication of Alzheimer's disease unlikely.

ZUSAMMENFASSUNG

Die Alzheimer Krankheit ist die am häufigsten auftretende neurodegenerative Erkrankung in den westlichen Industrieländern und zeigt eine steigende Prävalenz mit zunehmendem Alter (Bassiony, Rosenblatt et al. 2004; Potyk 2005). Die histopathologischen Merkmale dieser Erkrankung sind Aβ-Plaques und neurofibrilläre Tangles, sowie Neuronenverlust in den Gehirnen der Betroffenen. Zahlreiche epidemiologische Studien weisen auf einen zentralen Einfluss genetischer Faktoren hin, die zusammen mit Umwelt-, bzw. Lebensstilfaktoren einen Beitrag zur Alzheimer Krankheit leisten. Während Punktmutationen in drei Genen (*PS1, PS2* und *APP*) die autosomal-dominante Form der Erkrankung schon vor dem 60. Lebensjahr auslösen können, erhöht das ApoE ε4 Allel die Wahrscheinlichkeit mit zunehmendem Alter an Alzheimer zu erkranken. Weiterhin wurden in zahlreichen genetisch-epidemiologischen Studien signifikante Loci in verschiedenen chromosomalen Regionen identifiziert.

In dieser Arbeit wurde eine Feinkartierung von funktionellen und positionellen Kandidatengenen in vorher identifizierten Kopplungsregionen durchgeführt. Die ausgewählten SNPs wurden anhand eines Fall-Kontroll Kollektivs aus München genotypisiert und anschließend auf eine Assoziation mit der Alzheimer Krankheit getestet. Hauptaugenmerk wurde auf das Gen *APP* gerichtet, welches maßgeblich an der Ausprägung von AD durch die Bildung von Aβ beteiligt ist.

Insgesamt wurden 39 SNPs aus *APP* sorgfältig ausgewählt und anschließend mittels Matrix-Assisted Laser Desorption/Ionization Time Of Flight (MALDI TOF MS) genotypisiert. Die statistischen Auswertungen (Einzelmarker, Haplotypen und Sliding Window) der Genotypisierungsdaten ergab zwei chromosomale „hot-spot“ Regionen mit signifikanten Ergebnissen ($p < 0.05$), welche sich über 5 untersuchte SNPs in Intron 1; und 11 SNPs von Intron 3 bis Intron 8 erstrecken. Die am stärksten assoziierten SNPs wurden in einer weiteren unabhängigen Fall-Kontroll Studie aus Schweden, inklusiver einer gepoolten Auswertung, sowie in einem diskordanten Geschwisterpaarkollektiv aus Deutschland repliziert. Die Replikationsanalyse bestätigte eine Assoziation mit AD für acht SNPs (rs9941877, rs2830012, rs3787644, rs2070655, rs2830035, rs1041420, rs6516727 und rs2830099). Eine anschließende Assoziationsanalyse der gefundenen Genotypen aller 39 SNPs mit klinischen Größen (MMSE Punktzahl und Ausbruchsalter) lieferte ein positives Ergebnis für rs1783016 in Intron 14. Das Risikoallele (rs1783016-G) scheint mit einem späteren Erkrankungsalter, aber einer schnelleren Abnahme der kognitiven Fähigkeiten assoziiert zu sein. Nachfolgend wurde eine Auswirkung der vorher identifizierten Risikoallele

in post mortem isoliertem humanen Hirngewebe von Alzheimer Patienten und gesunden Kontrollhirnen untersucht, um funktionelle Auswirkungen der Polymorphismen auf transkriptionaler, translationaler und post-translationaler Ebene zu untersuchen. Das identifizierte Risikoallel T des SNPs rs1041420 aus Intron 3 zeigte eine erhöhte Genexpression von *APP*. Demnach würde das rs1041420-T Risikoallel einen Beitrag zur Ausbildung der AD durch erhöhte APP Substratmengen und nicht infolge überdurchschnittlicher Prozessierung leisten. Für die beiden SNPs rs6516727 und rs2830099 aus Intron 1 wurde jeweils eine Assoziation des vorher identifizierten Risikoallels (rs6516727-T und rs2830099-G) mit niedrigerem löslichen GesamtAβ-Gehalt und niedrigerem Aβ40-Gehalt gefunden. Die entsprechenden protektiven C-Varianten (rs6516727-C und rs2830099-C) scheinen eine Bildung der löslichen Aβ40-Form zu begünstigen, während Aβ42 in den betroffenen Hirnproben vermutlich bereits neuritische Plaques gebildet hat. Demnach würden die Risikoallele der beiden SNPs rs6516727 und rs2830099 möglichwerweise einen Beitrag zur Ausbildung von AD durch erhöhte APP Prozessierung und nicht infolge steigender Substratmengen leisten. Die angewandte Methode - die funktionelle Untersuchung der Einwirkung der assoziierten SNP Varianten auf die Entstehung von AD - sollte in einem größeren Kollektiv an Hirngewebsproben wiederholt werden. Insgesamt konnte nachgewiesen werden, dass verschiedene SNP-Varianten im *APP* Gen ein starker Risikofaktor für die Ausprägung der Alzheimer Krankheit sind.

Da die Proteine von *ADAM10* und *BACE1* durch ihre Funktion als α-, bzw. ß-Sekretase an der Prozessierung von APP und somit an der Entstehung von Aβ beteiligt sind, wurden 38 SNPs aus beiden Genen sorgfältig ausgewählt und analog zu APP genotypisiert und statistisch analysiert. Keiner der untersuchten SNPs aus *ADAM10* oder *BACE1* zeigte eine signifikante Assoziation mit AD. Deswegen wird angenommen, dass SNP-Polymorphismen in *ADAM10* und *BACE1* keinen wesentlichen Einfluss auf die Ausprägung der Alzheimer Krankheit haben.

Des Weiteren wurden zwei Gene (*ABCA1* und *LIPC*) untersucht, die am Lipidmetabolismus und somit am Aβ-Abbau und -Transport beteiligt sind. Von den 30 untersuchten SNPs aus *ABCA1* zeigten zwei (rs2525606 und rs2066720) eine sehr schwache Assoziation mit AD. Diese Ergebnisse konnten in der schwedischen Replikationsstudie jedoch bestätigt werden. Bei der folgenden Genexpressionsanalyse war das Risikoallel rs206676-G mit erhöhten *ABCA1* mRNA Levels in Hirnproben assoziiert, vermutlich durch erhöhte ApoE-Lipidation. Die 25 untersuchten SNPs aus *LIPC* zeigten keine replizierbare signifikante Assoziation mit AD, weswegen ein wesentlicher Einfluss von SNP-Varianten in *LIPC* auf die Ausprägung der Alzheimer Krankheit ausgeschlossen werden kann.

List of Abbreviations

Aß	Beta Amyloid
ABCA1	Member 1 of Human Transporter Sub-family ABCA
AD	Alzheimer's Disease, Morbus Alzheimer
ADAM10	Disintegrin Metalloproteinase
ADRDA	Alzheimer's Disease and Related Disorders Association
APP	Amyloid Precursor Protein
APO	Apolipoprotein
bp	Base Pairs
BACE1	Beta-site Amyloid Beta A4 Precursor Protein-cleaving Enzyme 1
CAD	Coronary Artery Disease
cCT	Craniale Computertomographie
cDNA	Complementary DNA
CERAD	Consortium to Establish a Registry of Alzheimer's Disease
Ch./Chr.	Chromosome
cMRT	Craniale Magnetic Resonance Tomography
Da	Dalton
DNA	Desoxyribonucleic Acid
dNTP	Desoxyribonukleosidtriphosphate
DSM	Diagnostic and Statistical Manual of Mental Disorders
EOAD	Early Onset Alzheimer's Disease
HDL	High-densitiy Lipoprotein
hME	Homogenous Mass Extension
HSD	Multiple Testing Using Tukey HSD = Honest Significant Difference
ICD	International Classification of Disease
LD	Linkage Disequilibrium
LDL	Low-densitiy Lipoprotein
LIPC	Hepatic Triglyceride Lipase
LOAD	Late Onset Alzheimer's Disease
LSD	Multiple Testing Using Tukey LSD = Least Significant Difference
M	Molar
MALDI TOF	Matrix-Assisted Laser Desorption/Ionization Time Of Flight
min	Minute
MMSE	Mini Mental Status Examination
mRNA	Messenger Ribonucleic Acid
MS	Mass Spectrometry
MUC	Munich Sample Collective
NFT	Neurofibrillary Tangles
NINCDS	National Institute of Neurological an Communicative Disorders
PCR	Polymerase Chain Reaction
PHF	Paired Helical Filaments
PS	Presenilin
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RTQ-PCR	Real Time Quantitative Polymerase Chain Reaction
SAP	Shrimp Alkaline Phosphatase
SF	Straight Filament
SNP	Single Nucleotide Polymorphism
S-TDT	Sib Transmissions-Disequilibrium-Tests
SWE	Swedish Sample Collective
TICS-M	Telephone Interview for Cognitive Status-Modified

Contents

1 Introduction

Dementias are the most common mental-health problems in the elderly (Jellinger, Danielczyk et al. 1990). According to the traditional definition, dementia (from latin „de“ = without, „mens“ = mind) is a secondary, advancing impairment of a formerly greater intellectual capacity (Rosler, Wichart et al. 2001). Dementia is not regarded as a specific syndrome, but rather as a genus for multiple cognitive deficits of the brain, which are caused by degenerative, vascular and trauma-caused disorders (Letenneur 2004).

The prevalence of dementias increases massively with advanced age (Bondy, Guo-Ross et al. 1998; Bassiony, Rosenblatt et al. 2004; Potyk 2005). Around the age of 60 intermediate and severe dementias play - with a prevalence of 1 % (Rosler, Wichart et al. 2001) - only a comparatively minor roll in industrial nations. Although in the age group between 70 and 74 years only ~3 % of individuals are affected, in the age group over 80 years, the percentage of affected persons increases up to 20 % (Letenneur 2004). Prevalence amongst the 95 – 99 year old even reaches 45 % emphasizing the age-dependency of the disease (Teri, McCurry et al. 1997). In Germany about 1 million people are suffering from a dementia (Forstl 2000; Bickel 2001). From these about 70% are suffering from Morbus Alzheimer (Alzheimer’s disease, AD) (Bickel 2000).

Due to the strongly reserved quality of life of affected people and enormous economic costs for care and therapy (Letenneur 2004), exploration of the course of disease and the development of effective methods of treatment are of utmost importance.

1.1 Alzheimer´s Disease: Definition and Introduction

Alzheimer´s disease was described for the first time by Alois Alzheimer in 1906 at a convention meeting in Tübingen. AD is characterized by a lingering beginning and a progressive course of disease, where cognitive impairments (affecting memory, power of judgment, intellectual power, language and learning), behavioral problems (disinhibition, euphoria, low frustration tolerance, emotional instability, crankiness, aggressiveness, changed sexual behavior) and an increasing loss of daily living skills occur (Forstl 2000). Additionally, unspecific accessory symptoms (depression, anxiety, misperception, delusion-like phenomenon, etc.) can appear. On the individual level, AD can proceed in varying degrees and does not follow a coarse division into single stages. The most commonly used diagnosis criteria are defined in the 10th edition of „International Classification of Disease“ (ICD 10), as well as in the 4th edition of „Diagnostic and Statistical Manual of Mental Disorders“ (DSM IV) of the American Psychiatric Association. Another concept was submitted by a collaboration of the workgroups of the „National Institute of Neurological and Communicative Disorders and Stroke“ (NINCDS) and the „Alzheimer’s Disease and Related Disorders Association“ (ADRDA). Diagnosis criteria are listed in the Appendix (§ 7.1).

Two different forms of AD can be distinguished – the sporadic form, occurring in ~75 % of the cases and the familial form (FAD, approx. 25 % of cases). Depending on the age of onset of the affected people the familial form is subdivided into two sub-forms: late-onset AD (LOAD) und early-onset AD (EOAD). LOAD occurs after the age of 65 and is prevalent in the majority of FAD-cases (Rademakers, Cruts et al. 2003). LOAD exhibits numerous non-Mendelian anomalies that suggest an epigenetic component in disease etiology (Wang, Oelze et al. 2008). The amount of patients suffering from the rarer EOAD is approx. 5 % (Kowalska, Pruchnik-Wolinska et al. 2004), where genetic influences seem to play a more significant role.

The severity of the disease can be defined by the individual’s ability of an autonomous conduct of life. With a weak severity an independent life is possible, but complex daily activities can not be conducted any longer. At an intermediate stage clear impairments of independent living occur and the affected person needs assistance. With a severe dementia a self-governing life is impossible.

Histopathological Markers

As the most important histopathological markers in the brain of AD-patients, neuritic plaques and neurofibrillary bundles (so-called "tangles") are presented (Fig. 1). Neuritic plaques are made of extracellular beta amyloid (Aβ) deposits (Verdile, Fuller et al. 2004). Neurofibrillary tangles are intracellular, abnormal (stretched or twisted) filaments, which consist primarily of a hyper-phosphorylated (Dickson 2004; Garcia and Jay 2004; Gomez-Ramos, Smith et al. 2004) and hyper-glycosed (Gong, Liu et al. 2005) form of the tau protein. Together with inflammatory occurrences in the brain, these histopathological markers contribute to the degeneration of neurons.

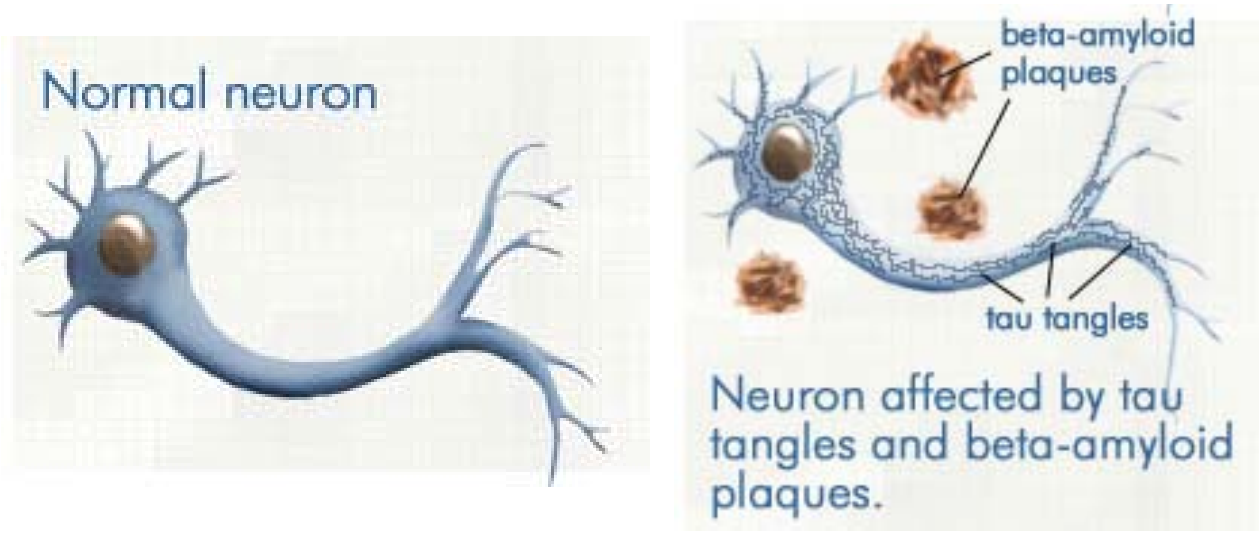

Fig. 1: Schematic of a normal and an AD affected neuron:
A normal neuron is shown on the left side. Communication between neurons in an AD affected brain (right side) is disrupted by beta-amyloid plaques and tau tangles. The pathological deposits cause degeneration of neurons, producing cell death and brain atrophy
(Garcia and Jay 2004; Gomez-Ramos, Smith et al. 2004; Verdile, Fuller et al. 2004).

According to the widely accepted „beta amyloid theory", the Aβ protein is the key molecule in AD pathogenesis (Hardy and Selkoe 2002; Sennvik, Bogdanovic et al. 2004; Verdile, Fuller et al. 2004). Aβ is produced by the so-called amyloidogenic pathway through proteolytic cleavage of the amyloid precursor protein (APP), a transmembrane glycoprotein (Cribbs, Poon et al. 2004; Verdile, Fuller et al. 2004). During the first step APP is cleaved by the β-secretase (BACE1, β-site cleaving enzyme). Thus, a soluble form of APP (APPsβ) and a C-terminal APP fragment (APP-CTFβ) are released (Verdile, Fuller et al. 2004). Thereafter, the enzymatic activity of the γ- secretase cleaves the APP-CTFβ molecule and Aβ is finally

generated (Sennvik, Bogdanovic et al. 2004). Gamma secretase is a multi-subunit protease complex, consisting of a catalytic sub-unit and three cofactors - Nicastrin (NCSTN), Anterior Pharynx Defective 1 (APH1A & APH1B) and the Presenilin Enhancer (PEN2) (Verdile, Fuller et al. 2004). The catalytic subunit of the γ-secretase is Presenilin 1 (PS1) or Presenilin 2 (PS2). The varying length of the Aβ protein is caused by the different γ-secretase-cleaving sites (Qi-Takahara, Morishima-Kawashima et al. 2005).

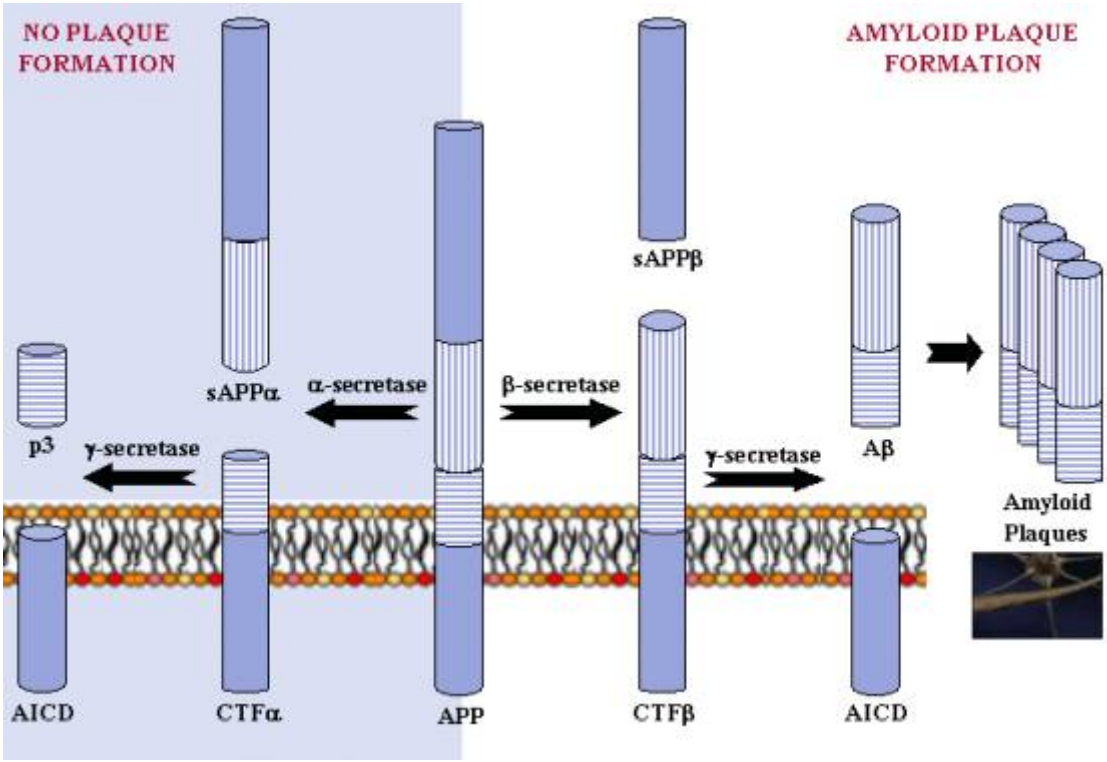

Fig. 2: Two possibilities of the proteolytic cleavage of APP:

The above described amyloidogenic pathway (right side) results through β- und γ-secretase activity in Aβ release and therefore enables forming of plaques. BACE1 was characterized as as β-secretase (Vassar, Bennett et al. 1999). The non-amyloidogenic (physiological) pathway (left side) is characterized by enzymatic activity of α-secretase, where APP is cleaved in the Aβ protein sequence (Postina, Schroeder et al. 2004). Thereby, the soluble carboxyl-truncated form of APP (sAPPα) and a C-terminal APP fragment (APP-CTFα) are released. In the next step, APP- CTFα is cleaved by γ-secretase and a non-amyloidogenic fragment (p3) is built. *ADAM 10*, *ADAM 17* und *MDC9* were described as α-secretase candidate (Postina, Schroeder et al. 2004; Verdile, Fuller et al. 2004; Postina 2008). The non-amyloidogenic pathway is also enabled by means of the protein kinase C (PKC) (Verdile, Fuller et al. 2004; Zheng and Koo 2006)

http://www.ebi.ac.uk/interpro/potm/2006_7/Page2.htm

With increasing length of the Aβ protein the tendency of self-aggregation and thus amyloidicity increases. Soluble Aβ proteins are 39 to 40 amino acids long and can also be detected in blood plasma and liquor of healthy individuals. These forms are not involved until later phases of the disease (Verdile, Fuller et al. 2004). However, proteins with a length of 41 – 43 amino acids (Aβ 41-43) are insoluble and interact, due to their beta-sheet structure, with each other and aggregate immediately. To a minor degree these proteins are also present in healthy individuals. They form hardly soluble or insoluble oligomers, so-called neuritic plaques. Neuritic plaques are characterized by a dense amyloid-core and can achieve a size up to 0.2 mm. In addition to Aβ, apolipoprotein (APO) E und J, α1–antichymotrypsin and proteoglycan appear in neuritic plaques (Dickson 2004). Intensified plaque formation involves loss of communication ability between different nerve cells. In almost all Alzheimer patients, amyloid sediments appear not only as plaques in the neurons, but also perivascular (Forstl 2000; Davis, Wagner et al. 2003). In these cases a reduced oxygen- and energy-supply of affected brain regions are the result, which can cause cerebral hemorrhage and strokes in advanced stages (Davis, Wagner et al. 2003; Melchor, Pawlak et al. 2003). In addition, pathological Aβ deposit may form an inflamed region, which in turn promotes the deposition of additional proteins (Combs, Bates et al. 2001).

The second histopathological AD marker is the formation of Alzheimer-fibrils or neurofibrillary tangles (NFT), which consists of paired helical filaments (PHF). In some cases, NFTs comprising PHF and SF (straight filament) or only SF are found. Both PHF and SF disturb the vital functions of neurons (Garcia and Jay 2004; Rissman, Poon et al. 2004). Although NFTs form inside of neuronal cells, extracellular tangles can be found as well. Apparently, the NFTs are released after cell death (Lovestone and Reynolds 1997). The major constituent of the NFTs is the hyper-phosphorylated and hyper-glycosylated tau protein (Garcia and Jay 2004; Rissman, Poon et al. 2004; Gong, Liu et al. 2005). In AD, Tau proteins are hyper-phosphorylated, due to an inbalance between kinases und phosphatases that affect Tau processing (Rissman, Poon et al. 2004). Due to an increased phosphorylation the protein is released from tubulin, a central part of microtubules. As a consequence, a stabilization of the cytoskeleton (Hartmann, Almeida et al. 2004), as well as axonal transport are inhibited (Garcia and Jay 2004). Because of its low solubility, released hyper-phosphorylated tau aggregates and forms NFTs or PHFs, respectively Tau is partly phosphorylated after NFT-building (Lovestone and Reynolds 1997). Overall, all of these pathological changes contribute to cell death. Besides phosphor-tau, ubiquitin can be present in NFTs (Rissman, Poon et al. 2004) and ApoE. Ubiquitin and ApoE are found in the cyst of the NFTs. Elevated phosphor-

tau-levels and NFTs can also be determined in patients which are suffering from other neurodegenerative diseases, e.g. fronto temporal dementia, vascular dementia, Creutzfeld-Jakob-disease, HIV-dementia, normal-pressure hydrocephalus, meningocephalitis, Lewy-Body-disease and corticobasal degeneration (Buee, Bussiere et al. 2000). Accordingly, phosphor-tau has no significant diagnostic value as specific AD marker, but can only be used in combination with other markers (Sergeant, Delacourte et al. 2005).

Both extra- and intracellular pathological sediments yield loss of brain substance (cerebral atrophy) and their density strongly correlate with the severity of the disease (Bauer, Hull et al. 1995). In comparison to same-aged healthy individuals, the weight of the brain of Alzheimer patients is significantly reduced, whereby different brain regions are not affected alike. In particular degradation of the cerebral cortex, especially the parietal-, temporal- and frontal lobe as well as the hippocampus formation are observed (Smith and Jobst 1996; De Leon, George et al. 1997; Nagy, Hindley et al. 1999; Pantel, Schonknecht et al. 2004). Neuronal cell death inhibits the transportation of material into healthy cells and also inhibits signal transduction pathways by means of neurotransmitters. The underrepresentation of acetylcholine is the most substantial neurotransmitter modification observed in AD patients, although it is not the only one. Eventually, a decreased acetylcholine level results in loss of memory, concentration and attention (Grossberg 2005). To a minor degree, concentrations of glutamate, noradrenaline, serotonin and several neuropeptides are reduced as well (Whitehouse 1987).

1.2 Risk Factors for Alzheimer´s Disease

AD is a complex, multi-factorial disease. It is influenced by lifestyle and environment dependent determinants as well as by genetic factors. The most frequently discussed risk factor for AD is age, with which the prevalence increases exponentially (Bassiony, Rosenblatt et al. 2004). According to estimations, 20 % of the population above 80 years is affected in western countries (Rosler, Wichart et al. 2001; Letenneur, Larrieu et al. 2004).

Intriguingly, the prevalence for LOAD in females is higher than in males (Colucci, Cammarata et al. 2006). One potential explanation for that phenomenon is the declined estrogen level or estrogen deficit during and after menopause, respectively (Balistreri, Grimaldi et al. 2006; Candore, Balistreri et al. 2006), as well as on average, a higher length of life of women (Corder, Ghebremedhin et al. 2004).

1.2.1 Lifestyle and Environment Dependent Risk Factors

Traumatic brain injuries, hyper-cholesterol anemia, as well as mental disorders were discussed as most important factors triggering AD (Grossman, Bergmann et al. 2006; Reid, Urano et al. 2007). Also complex, systemic diseases like diabetes mellitus (Gasparini, Netzer et al. 2002; Xu, Qiu et al. 2004) and hyperinsulin anemia (Luchsinger, Tang et al. 2004) were proven to be AD risk factors in several studies. Moreover, Itzhaki and coworkers showed, that the herpes simplex virus type 1 (HSV1) can contribute to development of AD in ApoE4 carriers (Itzhaki 2004; Itzhaki, Dobson et al. 2004).

Another aspect, which has often been discussed in combination with Alzheimer studies, was the education degree of patients (Karp, Kareholt et al. 2004; Colucci, Cammarata et al. 2006). Larger cognitive reserves and a life-long intellectual activity seem to delay the occurrence of dementia (Stern, Gurland et al. 1994; Qiu, Karp et al. 2003; Smyth, Fritsch et al. 2004).

In many clinical trials, an association between smoking and occurrence of AD was examined. Unlike few case-control-studies, where smoking was assumed to be a protective factor (van Duijn and Hofman 1991; Hellstrom-Lindahl, Mousavi et al. 2004), in most of the cohort-studies a negative influence on the occurence of AD was observed (Ott, Slooter et al. 1998; Juan, Zhou et al. 2004; Ott, Andersen et al. 2004)

The influence of alcohol consumption on appearance of AD was tested in numerous studies, too. Small doses of alcohol showed a potentially protective effect (Ruitenberg, van Swieten et al. 2002; Truelsen, Thudium et al. 2002; Letenneur 2004), whereas frequent alcohol consumption seems to increase the risk for AD (Luchsinger and Mayeux 2004). Moreover, redox-active metal-ions, especially Aluminium (Becaria, Campbell et al. 2002), copper (Rottkamp, Raina et al. 2001; Perry, Sayre et al. 2002) and iron (Rottkamp, Raina et al. 2001; Perry, Sayre et al. 2002), are discussed as potential triggers for the formation of plaques and tangles.

Although the above mentioned lifestyle- and environmental risk factors can be consistently detected after the onset of AD, the prediction of onset and course of disease, on basis of presence of the factors, are not very reliable.

1.2.2 Genetic Risk Factors

Growing prevalence of AD can mainly be ascribed to the increasing life expectancy in the population. Although lifestyle and environmental conditions influence quality of life and therewith development and course of disease, the probability of suffering from AD cannot just be explained with these factors. It seems to be more likely, that AD arises by reason of an interaction between several genetic factors and different environmental factors (Huang, Qiu et al. 2004).

Several genetic epidemiological studies gave indications on the influence of genes on the development of AD. Autosomal-dominant mutations in the *Presenilin 1* (*PS1*) gene on chromosome 14, in the *Presenilin 2* (*PS2)* gene on chromosome 1 and in the *Amyloid Precursor Protein* (*APP*) gene on chromosome 21 partly explain the significantly higher risk of onset for EOAD (Kowalska, Pruchnik-Wolinska et al. 2004; Zekanowski, Religa et al. 2004; Rebeck, LaDu et al. 2006). The pathological mutations in those three genes cause an increased Aβ production, especially of Aβ 42 (Ozturk, Minster et al. 2007). Nevertheless, in total, only a few hundred of families worldwide were reported to carry these mutations.

Up to now, only the ε4 allele of the *ApoE* gene was confirmed as a clear risk factor for LOAD (Rademakers, Cruts et al. 2003; Zekanowski, Religa et al. 2004). The *ApoE* gene is located on chromosome 19 and is found in three allelic variations, ε2, ε3 und ε4 (Mahley and Rall 2000). Inheritance of one or two ε4-alleles increases the dose-dependent risk of later occurrence of Morbus Alzheimer and moreover reduces the age of onset (Olarte, Schupf et al.

2006). Patients heterozygous for the ε4-allele sicken on average 4 years earlier, whereas homozygote patients with two copies of the risk allele are affected 6 – 8 years earlier than patients completely lacking the ε4-allele (Daw, Payami et al. 2000).

1.3 Genome-wide Search for Genetic Risk Factors

First informative data on disease-related chromosomal regions were originally provided by genome-wide linkage and association studies. The advantage of such genome-wide analyses is that no hypothetical assumptions concerning the pathophysiology of the disease is required.

In linkage studies, genetic markers that are distributed over the whole genome are determined within affected families and then genotyped. Micro-satellites serve as markers. Subsequently, the collective inheritance of these markers in families is investigated. A linkage between the marker allele and disease incidence is suggested when both occur together at a higher than average frequency. This approach enables the identification of chromosomal regions (so-called linkage regions), which are inherited in combination with the disease. In the next step – the so-called fine-mapping – the genes that are localized in the linkage region showing a probable involvement in the development of the disease are analyzed in detail (de la Chapelle and Wright 1998; Bleck, McGrath et al. 2001). Those loci are called positional candidate genes. For complex diseases with a late age of onset (e.g. Morbus Alzheimer), a linkage analysis is carried out for the affected individual and the siblings, due to the usually missing information of the parents. First significant linkage findings in EOAD families were reported for the chromosome-wide studies of the chromosomal regions Ch1q31-42 (Levy-Lahad, Wijsman et al. 1995), Ch14q24.3 (Nechiporuk, Fain et al. 1993; Cruts, Backhovens et al. 1995) and Ch21q21 (Goate, Haynes et al. 1989). Genome-wide linkage studies for LOAD pointed to several chromosomal regions (chromosomes: 1, 3, 5, 6, 9, 10, 12, 19, 21 and x) (Myers, Holmans et al. 2000; Lee, Cheng et al. 2006). Highest LOD (logarithm of the odds)-Scores, as a measure of the probability for linkage between selected markers and occurrence of the disease, were achieved for chromosomes 9, 10, 12 and 19 (Pericak-Vance, Bass et al. 1998; Kehoe, Wavrant-De Vrieze et al. 1999; Myers, Wavrant De-Vrieze et al. 2002; Blacker, Bertram et al. 2003).

The most applied current genetic research methods are based on genome-wide association studies. For this purpose, in a very large amount of subjects (>1000), approx. 500.000 SNP markers are genotyped in parallel on microarray-chips and subsequently tested for association with the disease. Genome-wide association studies are often carried out in a three-phase design. In the first phase (screening phase) selected SNPs are analyzed and the genotyping results are statistically evaluated. Significantly associated SNPs are subsequently validated in independent cohorts (phase two). In the third phase fine-mapping of the formerly analyzed region is carried out (Pearson, Huentelman et al. 2007). In all the genome-wide association studies that were performed up to now, the ApoE locus on chromosome 19 was confirmed as the most important genetic risk factor for LOAD (Coon, Myers et al. 2007; Reiman, Webster et al. 2007; Li, Wetten et al. 2008; Feulner, Laws et al. 2009). Amongst others, an association with AD was also reported for markers on chromosome 9 and 10 (Li H et al, 2008, Gruppe A et al. 2007). *GOLPH2*, *EBF3* und *SORCS1*, located in these chromosomal regions were identified as positional candidate genes. In our group, additional to the ApoE locus on chromosome 19, the top ten disease-associated genes, as presented from AlzGene´s version of 07-May-2008 (www.alzforum.org) were confirmed to be associated with AD. This finding demonstrates that genome-wide association studies are valuable for the identification of genetic variants associated with AD (Feulner, Laws et al. 2009).

Genome-wide association studies for EOAD have not been carried out up to now.

1.4 Candidate Genes for Alzheimer´s Disease

Preferentially, genes were analyzed, whose mutation could, according to nowadays knowledge, trigger the neuro-pathological process and therefore AD or could influence its course. Since former studies have proven a correlation between occurrence of AD and amount and presence of Aβ (Hardy and Higgins 1992), the main focus of the presented work was on the *APP* gene and the genes that are involved in the processing of APP. Additionally, genes involved in the lipid metabolism, such as the major genetic risk factor for AD, apolipoprotein E (ApoE) were taken into consideration during selection of candidate genes.

Moreover, results from genome-wide linkage studies were taken into account in the selection of candidate genes.

1.4.1 Amyloid Precursor Protein (APP)

The amyloid precursor protein (APP) is an integral membrane protein with a single membrane spanning region. It is expressed in many tissues and can be found concentrated in the synapses of neurons. It was assumed that it plays a decisive role in the formation and repair of synapses (Priller, Bauer et al. 2006) and neural plasticity (Turner, O'Connor et al. 2003), although its normal biological function has not been completely clarified up to now. The gene for APP (*APP*) is located on chromosome 21 (21q21) and contains at least 18 exons spanning 240 kilobases (Yoshikai, Sasaki et al. 1990; Lamb, Sisodia et al. 1993). Several isoforms of APP have been observed in humans, due to alternative splicing, ranging in length from 365 to 770 amino acids. Predominant transcripts are APP695 (exons 1 – 6 and 9 – 18), APP751 (exons 1 – 7 and 9 – 18) and APP770 (exons 1 – 13 and 14 – 18) (Ling, Morgan et al. 2003). Since certain isoforms seem to be preferentially expressed in neurons; changes in the neuronal ratio of these isoforms have been associated with Alzheimer's disease (Matsui, Ingelsson et al. 2007).

Due to APPs major role during AD pathogenesis, as described before in detail (see § 1.1), this gene is a major functional candidate gene.

Linkage studies have provided evidence for other susceptibility loci for late-onset Alzheimer's disease. One locus, on chromosome 21 (Myers, Wavrant De-Vrieze et al. 2002; Olson, Goddard et al. 2002; Blacker, Bertram et al. 2003), is of particular interest due to its characteristics of harboring one of the aforementioned genes, *APP*. Previously, coding mutations in *APP* have been associated with EOAD (Goate, Chartier-Harlin et al. 1991), however, this linkage region was largely observed in families with a later age of onset (Olson, Goddard et al. 2002). The APP gene represents a strong functional candidate for AD risk on many levels. Firstly, it is the parent molecule from which the core peptide of amyloid plaques, beta-amyloid (Aβ), is derived. Secondly, complete or partial trisomy of chromosome 21 leads to Down Syndrome including AD pathology only when the *APP* gene is present in three copies, suggesting that overexpression of *APP* may be a risk factor for LOAD (Rumble, Retallack et al. 1989; Prasher, Farrer et al. 1998). Furthermore, an *APP* locus duplication in trisomy 21 leads to elevated levels of circulating Aβ peptide (Schupf, Patel et al. 2001). Likewise, a recent study has reported that duplication of the *APP* locus is associated with a familial disorder that is characterized by dementia and cerebral amyloid angiopathy (CAA) (Rovelet-Lecrux, Hannequin et al. 2006). Taken together these observations suggest that APP

is a strong functional candidate for conferring risk for sporadic forms of AD with variability in APP expression providing the functional mechanism for this association. However, it is interesting to note that, despite this evidence, there are relatively few studies that have examined the role of APP as a risk factor in sporadic forms of AD.

A part from the study of Nowotny and colleagues (Nowotny, Simcock et al. 2007), where no association was observed in any of the 44 SNPs investigated, all previous studies have focused on a select number of polymorphisms, predominantly in the promoter region of the *APP* gene, and have to date presented inconclusive results. In a population of African American and Caribbean Hispanic ethnicity (Athan, Lee et al. 2002), the SNP rs459543 was presented to have an association in patients lacking the *ApoE* ε4 allele. Two other promoter polymorphisms rs466448 and rs11911934 have also been reported to influence the risk for LOAD by changing expression levels of *APP* (Lahiri, Ge et al. 2005). However, a subsequent study found no association of these SNPs, although an additional promoter polymorphism, rs463946, showed a weak association (Guyant-Marechal, Rovelet-Lecrux et al. 2007). Additionally, another study identified three rare variants in AD patients that, *in vitro*, showed neuron-specific increases in *APP* transcriptional activity (Theuns, Brouwers et al. 2006). Finally, Li and colleagues (Li, Perry et al. 1998) reported no association of a polymorphic tetra nucleotide repeat site, found in intron 7 of the *APP* gene, with LOAD.

Although *APP* is a strong functional and positional candidate, only one of the aforementioned studies (Nowotny, Simcock et al. 2007) used a fine mapping approach of SNPs across the entire gene. Therefore, a whole fine mapping approach of *APP* is of utmost importance to verify or replicate the former findings and to get new results for the genomic loci spanning the *APP* gene.

1.4.2 Candidate Genes Involved in APP Processing

Central to AD pathogenesis is the amyloid cascade hypothesis (Hardy and Higgins 1992)**.** Today, this hypothesis remains valid, except that it is less clear which form of beta-amyloid (Aβ), protofibrils or small oligomers, initiates the cascade. Key to this process is the consecutive cleavage of the amyloid precursor protein (APP) that occurs either through an amyloidogenic or a nonamyloidogenic pathway as described before (see Fig. 2 above). The amyloidogenic pathway results in the generation of beta-amyloid (Aβ) and is initiated through cleavage by the beta-site amyloid beta A4 precursor protein-cleaving enzyme 1 (BACE1).

The nonamyloidogenic pathway precludes the formation of Aβ through cleavage by alpha-secretase, an enzymes activity demonstrated in a disintegrin metalloproteinase, ADAM10.

1.4.2.1 Beta-site Amyloid Beta A4 Precursor Protein-cleaving Enzyme 1 (BACE1)

Neurotoxic Aβ is generated by two proteases; β-secretase, identified as the Beta-site amyloid beta A4 precursor protein-cleaving enzyme 1 encoded by *BACE1* at 11q23.2-q23.3, and the γ-secretase complex (Vassar, Bennett et al. 1999; Edbauer, Winkler et al. 2003).

The encoded protein is a member of the peptidase A1 protein family and a type I integral membrane glycoprotein of 70 kDa. It functions as an aspartic protease that is found mainly in the Golgi apparatus (Haniu, Denis et al. 2000). The *BACE1* gene contains 9 exons, producing four transcript variants encoding different isoforms: the full-length BACE1-501 and the three minor transcripts BACE1-476, BACE1-457 and BACE1-432 which are generated by in-frame alternative splicing. It was shown, that BACE1-457 and BACE1-476 isoforms showed weaker β-secretase activity than BACE1-501 (Tanahashi and Tabira 2001) or even no β-secretase activity at all (Ehehalt, Michel et al. 2002).

It was shown, that the low density lipoprotein receptor-related protein-1 (LRP1) not only interacts and traffics with APP, but also with BACE1 on the cell surface in association with lipid rafts. The BACE-LRP1 interaction leads to increased LRP1 extracellular domain cleavage and subsequent release of the LRP1 intracellular domain from the membrane. It was therefore concluded that LRP1 is a BACE1 substrate (von Arnim, Kinoshita et al. 2005).

Mice deficient in Bace1 seemed to be healthy, fertile, and appear normal in gross anatomy, tissue histology, hematology, and clinical chemistry according to investigations of Luo et al. They suggested that therapeutic inhibition of BACE1 for the treatment of Alzheimer disease may be free of mechanism-based toxicity (Luo, Bolon et al. 2001). In contrary, Willem and co-workers concluded from their studies that BACE1 is required for myelination and correct bundling of axons by Schwann cells (Willem, Garratt et al. 2006).

Whilst *BACE1* has been the subject of extensive study, these reports have focused on one or two exon 5 polymorphisms (for meta-anlaysis see the AlzGene database (Bertram, McQueen et al. 2007)) with only one study utilizing a whole-gene approach (Todd, McKnight et al. 2008).

BACE1 represents, due to its involvement in APP processing, a prime functional candidate for AD. Furthermore, the location of *BACE1* in the vicinity of suggestive AD

linkage peaks at 11q25 (Blacker, Bertram et al. 2003), also makes this gene a positional candidate.

1.4.2.2 Disintegrin Metalloproteinase (ADAM10)

An alternative cleavage pathway, involving α-secretase, precludes the formation of Aβ (Kojro and Fahrenholz 2005). ADAM10, a member of the ADAM (a disintegrin and metalloproteinase) family of proteinases, which is encoded by the *ADAM10* gene on chr 15q21-q23, has been shown to exhibit this α-secretase activity (Lammich, Kojro et al. 1999).

The protein is a cell surface protein with a unique structure, possessing both potential adhesion and protease function. The *ADAM10* gene contains 16 exons, 15 introns and covers about 160 kb (Prinzen, Muller et al. 2005). The protein contains a N-terminal signal sequence, followed by a prodomain, a metalloprotease-like domain, a disintegrin-like domain, a cysteine-rich region, an epidermal growth factor (EGF)-like repeat, a transmembrane domain, and a C-terminal cytoplasmic tail (Wolfsberg, Primakoff et al. 1995).

It was shown in mice that carry a human APP mutation, that moderate neuronal overexpression of human *ADAM10* increased the secretion of the neurotrophic soluble APP domain, which is released through alpha-secretase-activity. In addition, formation of amyloid beta peptides was reduced and their deposition in plaques prevented. Expression of mutant catalytically-inactive ADAM10 led to an enhancement of the number and size of amyloid plaques in the brain (Postina, Schroeder et al. 2004).

Up to now, polymorphisms in *ADAM10* have not received much attention in the literature (Prinzen, Muller et al. 2005). *ADAM10* represents, due to its involvement in APP processing, a prime functional candidate for AD. Furthermore, the location of *ADAM10* in the vicinity of suggestive AD linkage peaks at 15q22 (Scott, Hauser et al. 2003), also makes it a positional candidate.

1.4.3 Candidate Genes Involved in Lipid Metabolism

In addition to the aforementioned loci, genes involved in the lipid metabolism, such as the major genetic risk factor for AD, apolipoprotein E (ApoE), were taken into consideration during selection of candidate genes for this study.

1.4.3.1 Member 1 of Human Transporter Sub-family ABCA (ABCA1)

ABCA1 is a membrane associated protein. It belongs to the ATP-binding cassette (ABC) transporters from sub-family A. Santamarina-Fojo and colleagues found that the ABCA1 gene spans 149 kb and contains 50 exons (Santamarina-Fojo, Peterson et al. 2000) and is located on chromosome 9 (9q31.1) (Luciani, Denizot et al. 1994).

ABCA1 functions as a cholesterol and phospholipid efflux pump in the cellular lipid removal pathway. It was suggested that ABCA1 promotes cholesterol and phospholipid efflux by directly transporting both lipids as substrates. The ABCA1 gene expression is markedly increased in cholesterol-loaded cells. Upregulation of ABCA1 activity is known to form atheromatous deposits (plaques), especially on the innermost layer of arterial walls (Wang and Tall 2003).

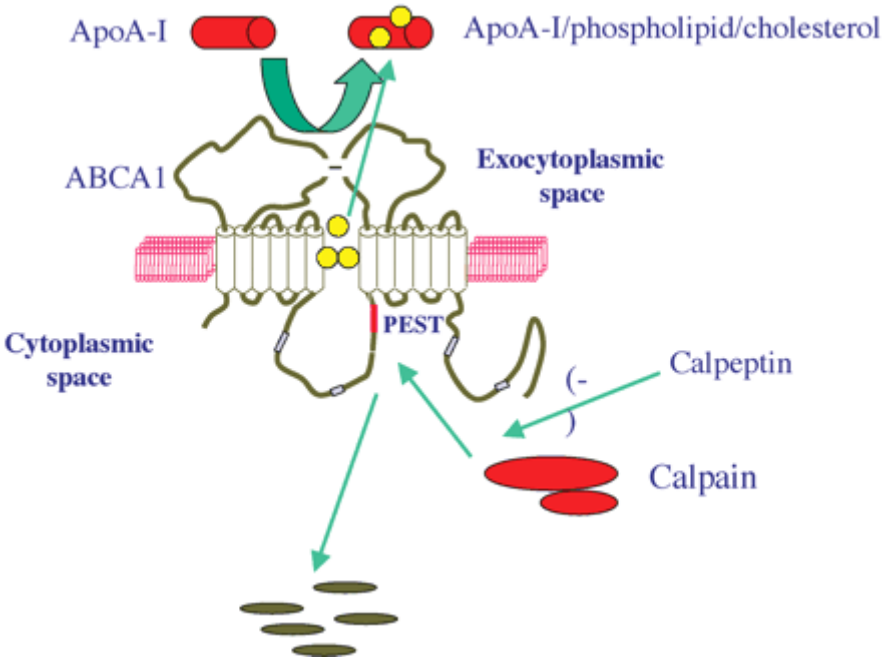

Fig. 3: Regulation of ABCA1-mediated cholesterol efflux

ABCA1 performs a cellular cholesterol and phospholipid efflux. The substrates are donated to lipid-poor apolipoproteins. ApoA-I, the major apolipoprotein component of HDL, promotes ABCA1-mediated cholesterol and phospholipid efflux, probably by directly binding to ABCA1. ABCA1 contains a PEST-proline (P), glutamate (E), serine (S), and threonine (T)-sequence in the intracellular segment. PEST causes ABCA1 degradation by a thiol protease, calpain. ApoA-I and apoE stabilize ABCA1 in a novel mode of regulation by decreasing PEST sequence-mediated calpain proteolysis.

(Wang and Tall 2003)

The above described mechanism is the rate-limiting step in the production of nascent high-densitiy lipoprotein (HDL) (Oram and Lawn 2001). The finding, that in AD the lipid mechanism is likely disturbed, is supported by different findings: its genetic association with ApoE (Strittmatter, Weisgraber et al. 1993), the occurence of reduced HDL cholesterol levels in AD patients (Oram and Lawn 2001), and the observation that cholesterol influences Aβ metabolism (Sparks, Scheff et al. 1994). Moreover, it was shown that *ABCA1* expression can directly affect Aβ levels (Koldamova, Lefterov et al. 2003). A mouse-model proved that overexpression of ABCA1 reduces Aβ deposits in the brain (Wahrle, Jiang et al. 2008). It was also shown, that deficiency of ABCA1 leads to the loss of approximately 80% of apoE in the brain, and the residual 20% that remains is poorly lipidated. Conversely, robust overexpression of *ABCA1* in the brain promotes apoE lipidation and nearly eliminates the formation of mature amyloid plaques (Hirsch-Reinshagen, Burgess et al. 2008).

Mutations in *ABCA1* have been demonstrated to be associated with Tangier disease and a familial high-density lipoprotein deficiency (Bodzioch, Orso et al. 1999; Brooks-Wilson, Marcil et al. 1999; Rust, Rosier et al. 1999). Tangier disease is characterized by dramatically lowered HDL and APOA1 concentrations as well as lowered cellular cholesterol efflux. This results in an increased risk for premature coronary artery diseases. Merched and coworkers have also observed reduced APOA1 levels in AD patients (Merched, Xia et al. 2000). All of that makes *ABCA1* to a potential functional candidate gene.

Genome-wide linkage studies for LOAD pointed to several chromosomal regions (compare § 1.3). The highest LOD-Scores were associated with loci on chromosomes 9q and 10q (Pericak-Vance, Bass et al. 1998; Kehoe, Wavrant-De Vrieze et al. 1999; Bertram, Blacker et al. 2000; Myers, Wavrant De-Vrieze et al. 2002; Blacker, Bertram et al. 2003). The location of *ABCA1* on chromosome 9q, which is approximately 5 mb distal from previously identified loci linked with AD, makes the gene also to a potential positional candidate gene (Pericak-Vance, Bass et al. 1998; Kehoe, Wavrant-De Vrieze et al. 1999; Bertram, Blacker et al. 2000; Myers, Wavrant De-Vrieze et al. 2002; Blacker, Bertram et al. 2003).

Studies investigating the association of genetic variants of *ABCA1* with an increased risk for AD were performed before, but came out with contradictive findings. Moreover, most of the studies only investigated selected SNPs (1 – 5) from the promotor or the coding regions (Wollmer, Streffer et al. 2003; Katzov, Chalmers et al. 2004; Kolsch, Lutjohann et al. 2006; Shibata, Kawarai et al. 2006; Rodriguez-Rodriguez, Mateo et al. 2007; Wang and Jia 2007), or some isolated SNPs (9 and 10) that do not span the whole gene (Li, Tacey et al. 2004; Wahrle, Shah et al. 2007). Up to now only one study used an approximate fine-mapping

approach, however only including 19 SPNs in a Chinese cohort (Chu, Li et al. 2007). Therefore, a whole fine mapping approach of *ABCA1* is of utmost importance to verify the former findings and to achieve new findings over the entire gene locus.

1.4.3.2 Hepatic Triglyceride Lipase (LIPC)

Hepatic lipase that is predominantly expressed in the liver is also known as hepatic triglyceride lipase (LIPC) and is encoded by the *LIPC* gene. LIPC functions as a triglyceride hydrolase and as a ligand/bridging factor for receptor-mediated lipoprotein uptake.

LIPC is located on chromosome 15 (chr15q21 – q23) and contains 9 exons that span over 35 kb (Cai, Wong et al. 1989).

Hepatic lipase plays a major role in the regulation of plasma lipids. The same function had been shown for lipoprotein lipase (LPL) and lecithin cholesterol acyltransferase (LCAT) (Cai, Wong et al. 1989). LPL and HL can be found at the endothelial surfaces of extra-hepatic and hepatic tissues, respectively. Already small deficiencies of one of these enzymes have been identified to be associated with pathologic levels of circulating lipoprotein particles.

Variants in *LIPC* had previously been associated with elevated LDL or lowered HDL levels. (Kathiresan, Melander et al. 2008).

As mentioned before, sporadic or late onset Alzheimer's disease is a complex neurodegenerative disease that is associated with a variety of genetic and environmental risk factors. However, to date, only one undisputed genetic component has been identified, being the ε4 allele of ApoE (Saunders, Strittmatter et al. 1993; Martins, Clarnette et al. 1995). As a starting point for further genetic studies in AD, disorders intimately linked with ApoE have been investigated as a source of candidate genes. Besides AD, ApoE is also a strong genetic risk factor for increased LDL-cholesterol and coronary artery disease (CAD) (Baroni, Berni et al. 2003; Martins, Hone et al. 2006). As such, genes, or more specifically single nucleotide polymorphisms (SNP)s, that modulate cholesterol homeostasis provide important functional candidates for AD association studies (Wollmer, Sleegers et al. 2007). One such example is Hepatic lipase (LIPC, also known as hepatic triglyceride lipase) where several SNPs are associated with altered cholesterol concentrations and CAD (Baroni, Berni et al. 2003; Knoblauch, Bauerfeind et al. 2004).

Since LIPC is associated with altered lipid metabolism, much like the major genetic risk factor for Alzheimer's disease - apolipoprotein E - this gene is a potential functional candidate for AD risk.

1.5 Objective

Morbus Alzheimer is the most frequently identified cause of dementia in advanced age. With increasing prevalence this disease is the fourth most frequent cause of death in industrialized countries. Many studies prove that besides external trigger factors (environmental conditions and life style), the genetic susceptibility also influences the course of disease. In addition to the currently known and probably most important candidate genes *PS1*, *PS2*, *APP* and *ApoE,* further linkage regions could be identified during genome-wide studies.

Aim of the present study is identification of candidate genes due to their function and correlation with the above mentioned candidate genes and their location in the known linkage regions and finally their functional characterization. Therefore a contribution to the investigation of the molecular fundamentals of AD pathogenesis is supposed to be made. Correspondingly, the selection of the candidate genes was focused on these genes, whose modification could influence the development of the disease. Further selection criterion for candidate genes was their chromosomal localization.

For the selected candidate genes noted SNPs are searched in public databases. The newly identified SNPs are thereafter genotyped by means of MALDI TOF MS in a case-control cohort from Munich (as specified in the chapter below) and tested for an association with AD. Positive association signals are validated in other independent case-control, as well as sibling samples. Consecutively, strongest association results are to be investigated in appropriate functional studies.

2 Methods

2.1 Phenotyping of Subjects - Neuropsychological Tests

All subjects were undertaken detailed psychiatric, neurological, neuropsychological and clinical examinations. They included test of cognitive performance via standardized neuropsychological survey, self-anamnesis and medical history from important others, cranial imaging (cCT or cMRT), neurological findings and routine laboratory examinations (including determination of vitamin B12, folic acid, thyroid parameters, lyme serology and TPHA, etc.).

For diagnosis of dementia the criteria of ICD-10 (see § 7.1.1) were applied. The clinical diagnosis of probable AD was established according to the NINCDS-ADRDA (National Institute of Neurological and Communicative disorders and Stroke-Alzheimer's Disease and Related Disorder Association) criteria (see § 7.1.3).

The control group was matched for age, gender, geographical location and ethnicity and consisted of cognitively healthy subjects.

Standardized neuropsychological tests represent the most differentiated level of diagnostic findings. There are normal values for population random samples, so that test results of potential patients can be compared to those from healthy subjects of same age, gender and educational level. For diagnosing within the scope of this study Mini Mental Status Examination (MMSE) and Telephone Interview for Cognitive Status-modified (TICS-M) tests were performed.

2.1.1 Mini Mental Status Examination (MMSE)

Mini Mental Status Examination (MMSE) test (Folstein, Folstein et al. 1975), also known as Folstein-Test (after the author Marshal F Folstein), is the most common screening test for survey of cognitive disorders. It only complements neuropsychological examinations and can not be used for diagnosing different forms of dementia. MMSE contains 30 items, which are subdivided into 5 categories (retentiveness, commemorative skills, orientation, concentration and language). However it is impossible to check complex cognitive functions with this test.

Analysis is performed by simple addition of the points given. Cognitive healthy subjects achieve results between 24 and 30 points. Subjects with light cognitive disabilities achieve 20 to 23 points. A dementia is assumed below 20 points. Cases with less than 10 points are called severe dementia. It has to be bared in mind that there is a correlation between the obtained points and the educational level. Light cognitive restrictions of subjects with higher intelligence or good education are hard to diagnose. Those are suspected of having cognitive defect from 28 points and below. On the other hand MMSE test delivers false positive results for subjects with lacking education (De Jager, Hogervorst et al. 2003). To avoid that within this work control subjects with less than 28 points were excluded from the study (Riemenschneider, Blennow et al. 2006).

Due to the easy practical performance (duration of the test ca. 10 min.), MMSE is applied in many standardized test batteries and big epidemiological studies.

2.1.2 Telephone Interview for Cognitive Status-Modified (TICS-M) Test

The Telephone Interview for Cognitive Status (TICS) Test (Brandt J et al., 1988) is a telephone version of the MMSE test and strongly correlates with the written form (Jarvenpaa, Rinne et al. 2002); (Jorm, Scott et al. 1988).A modified version of the TICS test (TICS-M) was applied in this study. TICS-M includes the questions of the original TICS in terms of orientation, concentration, memory, calculating, attention, language and conceptual thinking. Additionally to the immediate recall of a list of words TICS-M contains a delayed recall. In this part of the test verbal comprehension and practical are tested.

The TICS-M test consists of 21 items and takes 6 to 8 minutes. Duration of education was taken into account for evaluation and the overall result was corrected accordingly. The maximum score is 50 points, at which a higher number of points refers to a better achievement.

2.2 Selection of SNPs

Influence of genetic risk factors on the progression of the disease was tested by means of SNP association analysis. SNPs are variations of single base pairs in DNA-strands, which occur approx. every 200 bp in biallelic form (Rostoks, Mudie et al. 2005). SNPs were selected preferentially within coding/exon regions, promotor regions and TFBS (Transcription Factor Binding Sites). SNPs causing changes in the amino acids (missense mutations) were preferred. For the performed association analysis biallelic SNPs from public databanks (see § 3.2.6) were chosen, analyzed through MALDI TOF MS genotyping and subsequently examined for associations with AD. Only validated SNPs with a minor allele frequency of at least 5% were taken for analysis.

2.3 Isolation of Nucleic Acids

2.3.1 DNA Isolation

DNA was isolated from whole blood with Puregene Genomic DNA-Isolation Kits (Gentra Systems, Minneapolis, USA). Acaryote erythrocytes were isolated from cells with intact nuclei by selective lysis and subsequent centrifugation. Thereafter lysis of the cells containing nuclei was performed after addition of a DNA-stabiliser. Furthermore, proteins were eliminated by a salt precipitation reaction. Precipitation of the solved genomic DNA from the supernatant was carried out using 100% isopropanol. Precipitated DNA was then washed with 70% ethanol and the dried pellet re-solved in 1-2 mL TE-buffer over night. On average 300 – 400 µg genomic DNA could be isolated from 9 – 10 mL whole blood with this method. Stock DNA solutions were stored in single tubes at 4°C. For further details see the kit instruction.

2.3.2 RNA Isolation

Unlike DNA, RNA is very instable. Therefore it is of most importance, to separate the stable RNases as fast as possible from the samples. RNA isolation from brain tissue was carried out with RNeasy® Lipid Tissue Mini Kit (Qiagen GmbH, Hilden, Germany). At first brain tissue was homogenized in QIAzol Lysis Reagent. It consits of guanidiniumthiocyanat (GTC) and phenol. GTC effects lysis of the cells and solution of the RNA. A high GTC concentration enables a selective precipitation of the cellular proteins, while the RNA remains in solution. At the same time phenol inactivates the RNases. After addition of chloroform and subsequent centrifugation, the mix separates into 3 phases. RNA is in the upper colorless watery phase, the genomic DNA in the lower reddish organic phase. The denaturated proteins form a insoluble interphase. Isopropanol is added to the watery phase, precipitating the RNA. Afterwards RNA is bound to the membrane of the Mini Spin Column. Salt and isopropanol carryovers are removed by washing with 75% ethanol. Isolated RNA was dissolved in nucleasefree water and stored in single tubes at -80°C. For further details see the kit instruction.

2.4 Determination of Concentration of Genomic DNA and RNA in Solutions

Concentrations of DNA samples dissolved in water can be determined due to absorption of the nucleic acids at 260 nm. Artifacts from precipitation, derived from isolation of genomic DNA, also show maximum absorptions at 260 nm. Measured values for double stranded (ds) DNA are mainly falsified by single stranded (ss) DNA and RNA as well as single nucleotides. For calculation of the purity of the DNA, samples will be measured in the photometer at 280 nm as well. The ratio between the extinctions at 260 nm and 280 nm are taken to evaluate the purity of the samples. Pure DNA has a A260/A280 ratio of 1.8. Values below 1.8 shows contaminations with proteins, values above 2.0 are a sing of impurities from RNA (Lottspeich 1998).

Purity of RNA is analyzed photometric at λ=260 nm and λ=280 nm accordingly. A ratio of 2.0 proves a clean RNA isolation (Lottspeich 1998).

2.5 Test of DNA and RNA for Degradation

Before starting with genotyping, the DNA was examined for possible degradations. For that purpose 1 µL of each undiluted sample that was resolved in 1xTE buffer was brought onto a 0.8% gel. A single and clear compression band after staining with ethidium bromide proved no degradation. A 10000 – 500bp marker was used (Bio-Rad Lab. GmbH, München, Germany).

Quality of the RNA was tested on a 1.2% denaturating gel. For avoidance of hydrolytic activity of the RNases, the gel was prepared with DEPC water. Formaldehyde was used as denaturating agent. The aldehyde groups of the formaldehyde form Schiff´s bases with the amino groups of the nucleotide bases. Thus formation of intra- and intermolecular hydrogen bonds was inhibited and the RNA remains linear. On such a gel the complete intact RNA forms defined, clear 28S- and 18S-rRNA bands. The 28S-rRNA bands (5.1 kb for human RNA) are approx. double as intensive than the 18S-rRNA bands (1.9 kb for human RNA). The weak fluorescence between both bands and under the smaller band comes from mRNA. A 10000 – 500 bp marker was used (Bio-Rad Lab. GmbH, München, Germany).

2.6 Allocation on the Plates

The DNA, which was onward genotyped via MALDI TOF MS, was diluted to a final concentration of 100 ng/µL and transferred into the wells of 96 microtiterplates. For preparation of the 384 microtiterplates the DNA was diluted to 3.33 ng/µL. DNA from four 96 microtiterplates was combined on one 384 microtiterplate (5 µL per well) by means of the Tecan pipetting robot (Tecan AG, Crailsheim; Germany). For a later test of the quality of genotyping at least 10 DNA samples were applied in duplicate on each 384 microtiterplate and compared after analysis.

2.7 DNA- and RNA-Electrophoresis

Nucleic acids migrate to the anode of an electric field, due to its negative charge at the phosphate diester group. The speed of movement is indirect proportional to the molecular mass of the molecule. Depending on the size of the DNA and RNA fragments that were examined, 0.8 – 3 % agarose gels were prepared. Agarose was given in electrophoresis buffer (1x TBE buffer) and solubilized by heating. Ethidium bromide is added to the assay (end concentration of ~0.2 µg/mL). It intercalates in AT-rich sequences of the nucleic acid and makes it visible under UV light (302 nm).

2.8 Polymerase Chain Reaction (PCR)

The Polymerase Chain Reaction (PCR) (Saiki, Gelfand et al. 1988) was developed in the 80s by Dr. Kary B. Mullis. This method enables synthesis of a high number of copies of specific DNA sequences and cDNA sequences, respectively. During PCR DNA is amplified enzymaticly *in vitro*. A PCR consists of three main steps (denaturation, hybridization and elongation), which are repeated 30 to 45 times.

The reaction starts with thermic denaturation (95°C) of the dsDNA, where complementary strands are divided. After denaturation the reaction mixture is cooled down. During decrease of temperature super concentrated primers (oligonucleotides) can align to the now single stranded DNA matrices. Primer sequences are designed so that they can align to the sense strand (anti-sense primer) as well as to the anti-sense strand (sense primer). Those

types of oligonucleotides are called antiparallel primers. Hence a primer is aligned to each ssDNA strand and the 3'-hydroxyl group of the primer will be beginning of the nascent (extending) DNA strand. After addition of DNA polymerase and all four dNTPs a new DNA strand complementary to the DNA template is synthesized *in vitro* during elongation step. Taq polymerase is used for extension, an enzyme isolated from the thermophilic bacterium *Thermus aquaticus*. The enzyme has its optimum activity temperature at 72°C. Since this polymerase is not inactivated at temperatures above 90°C, the PCR can be carried out cyclical in a periodically heat- and coolable metal block uninterrupted.

After the first cycle a total of two partially doublestranded DNA strands are existent. Single repetition of the process delivers 4 copies of both template DNA strands. From the second cycle on the amount of replicated peaces increases exponentially.

The amplification was carried out with PCR equipments from MJ Research (Boston, USA) and Applied Biosystems (CA, USA) under the following conditions:

Tab. 1: Standard temperature profiles for PCR

Time:	Temperature:	Step:
15 min	95°C	Initialization
20 sec	95°C	Denaturation
30 sec	X°C	Annealing
1 min	72°C	Elongation / Extension
3 min	72°C	Final elongation
Permanent	4°C	Final hold

Steps Denaturation, Annealing and Elongation were repeated 45 times (45 cycles). Annealing temperature was adjusted for each assay and ranged between 56°C and 60°C.

In the scope of this work those genomic regions, where SNPs are located, were amplified by means of the above described PCR method. For obtaining a high PCR-efficiency, primers were designed so that PCR products were 80 to 120 bp. Sense and antisense-primer which were used for MALDI TOF MS analysis contained besides the sequence specific region an additional motive of 10 bases. This motive, the so-called “Tag”, fulfills several tasks. Firstly the amplification reaction is processed more evenly, when more PCR reactions are carried out in the same reaction tube. Secondly, mass of these primers has to be increased by means of these “Tags” insofar that they will not disturb subsequent MALDI TOF MS analyses. Since spare primers, which were not used up during PCR, are not removed during homogenous mass extension (hME), they are outside the mass region of the primer-extension product.

PCR reactions, which were part of the MALDI TOF MS genotyping method, were conducted in a reaction volume of 5 µL in 384 micro titer plates. Dried DNA was present in each well. Prepared master mix was pipetted into the wells by means of robotic workstation Genesis (Tecan GmbH, Crailsheim, Germany). The master mix was prepared according to the following scheme:

Tab. 2: Pipetting scheme for PCR master mix

total volume of 5 µL and 5 µL dried DNA (1 ng/µL)

Menge:	Substance:	Concentration:
2.850 µL	H_2O	
0.625 µL	10x buffer containing gelatine	
0.100 µL	dNTPs	25 mM dATP, dCTP, dGTP, dTTP each
1.000 µL	Primer mix (forward and reverse)	10 pmol/µL each
0.325 µL	$MgCl_2$	25 mM
0.100 µL	Hotstar Taq	5 U/µL

All used primers and lengths of individual PCR-fragments are reported in the Appendix.

2.9 iPLEX Reaction

The iPLEX reaction is a universal method for the detection of the different alleles of a SNP in formerly amplified DNA. The iPLEX process is subdivided in 4 main steps, which are described in detail below:

2.9.1 SAP Reaction

Before the properly iPLEX-reaction, the shrimp alkaline phosphatase (SAP)-reaction is performed. Surplus dNTPs from the PCR are cleaved by addition of this enzyme. This proceeding is necessary, since free nucleotides would generate other primer extension products, which would disturb the correct SNP detection. The SAP master mix was prepared according to the following scheme:

Tab. 3: Pipetting scheme for SAP master mix

2 µL per well

Volume for single reaction:	Substance:	Concentration:
1.53 µL	H_2O	
0.17 µL	SAP buffer	10x
0.30 µL	SAP enzyme	1 U/µL
7.00 µL	PCR-product	

and were carried out under the following conditions:

Tab. 4: Conditions for SAP reaction

Time:	Temperature:	Step:
40 min	37°C	Incubation
5 min	85°C	Deactivation of SAP
permanent	8°C	Cooling

2.9.2 Extension Primer Adjustment

Due to the fact, that the highest mass primer (~8500 Da) has a 25% lower peak intensity than the average of the lower mass primers, a primer adjustment to equilibrate signal-to-noise ratios is required. Low mass primers were concentrated higher to achieve equal peak intensity. The applied method to adjust high plex extension primers is to divide the primers into four groups, where the first group of low mass primers are mixed at 625 nM, the following group at 830 nM, the third group at 1.04 µM, and the higher mass group at 1.25 µM final reaction concentration 9 µl as shown below. This proceeding is important to achieve high call rates and good accuracy.

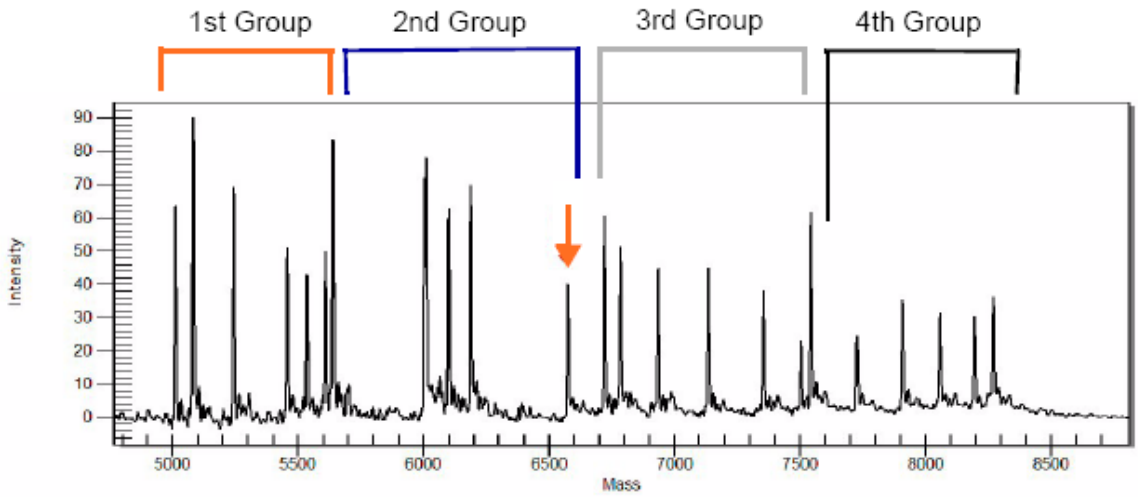

Fig. 4.: Primer extension adjustment according to the double 4-step method

High mass primers show a higher peak intensity than low mass primers. To achieve similar peak intensities, primers are devided in to 4 groups according to their mass and are thereafter concentrated accordingly in the subsequent iPLEX reaction (SEQUENOM).

2.9.3 iPLEX Reaction

Subsequently, the iPLEX reaction was performed. Therefore an iPLEX reaction mix (primer, enzyme, buffer, termination mix) is added. Extension-Primers are designed that they end exact one base before the SNP to be analyzed. In the reaction mixture, all four mass-modified nucleotides—A, T, C, and G—are present. During the iPLEX reaction, the primer is extended by one of the nucleotides, which terminates the extension of the primer. Thus, allele-specific extension products are generated, varying in length and mass depending on the present allele.

iPLEX reaction was carried out according to the following instructions and conditions:

Tab. 5: Pipetting scheme for iPLEX master mix

2 µL per well

Volume per reaction:	Substance:	Concentration:
0.755 µL	H_2O	
0.200 µL	iPLEX buffer	10x
0.200 µL	iPLEX termination mix	
0.804 µL	Primer mix* 0.625 : 1.25 µM	
0.041 µL	iPLEX enzyme	
9.000 µL	PCR-Product (+SAP-Mix)	

Tab. 6: Conditions for iPLEX -reaction

40 cycles were performed, where Hybridization and a short heat-up were repeated 5 times within each cycle

Time:	Temperature:	Step:
30 sec	94°C	Initial denaturation
5 sec	94°C	Denaturation
5 sec	*52°C*	*Hybridization*
5 sec	*80°C*	*Short heat-up*
3 min	72°C	Elongation
permanent	8°C	Cooling

All serial pipetting steps were conducted by the pipetting workstation Multimek96 Automated Channelpipettor (Beckmann Coulter; Fullerton, USA) by means of programmed standard procedures (Sequenom, Hamburg, Germany). Thermocycling for SAP- and iPLEX-reactions was carried out on PCR cyclers from MJ research und BA Gene under standard conditions (Manual Processing Procedure for the MassARRAY II System, Sequenom Hamburg, Germany).

2.9.4 Cleaning up of iPLEX Reaction Products and Nanodispensing

After elongation reaction an ion exchange resin (Spectro-CLEAN, Sequenom, Hamburg, Germany) was added to avoid adsorption/attachment of cations. Nucleic acids show a high affinity to alkali and alkaline earth ions. These cations disturb the MALDI TOF MS-analysis. The emerging single signals, which are attached to the actual molecule ion, cause a reduction of the signal-noise-ratio. Purification was carried out according to the standard protocol from Sequenom (Manual Processing Procedure fort he MassARRAY II System, Sequenom Hamburg, Germany).

iPLEX-products were spotted onto a 384 silicon chip by means of a nanoliter spotter. The chip is covered with a crystalline (3-Hydroxypicolinacid). Besides the 384 matrix spots, onto which the samples are transferred, 10 additional matrix spots are located on the chip. Onto these chips a calibrant (Sequenom Hamburg, Germany) spotted, containing a mixtrue of 3 oligonucleotides of known masses and serves for calibration of the analyzing system.

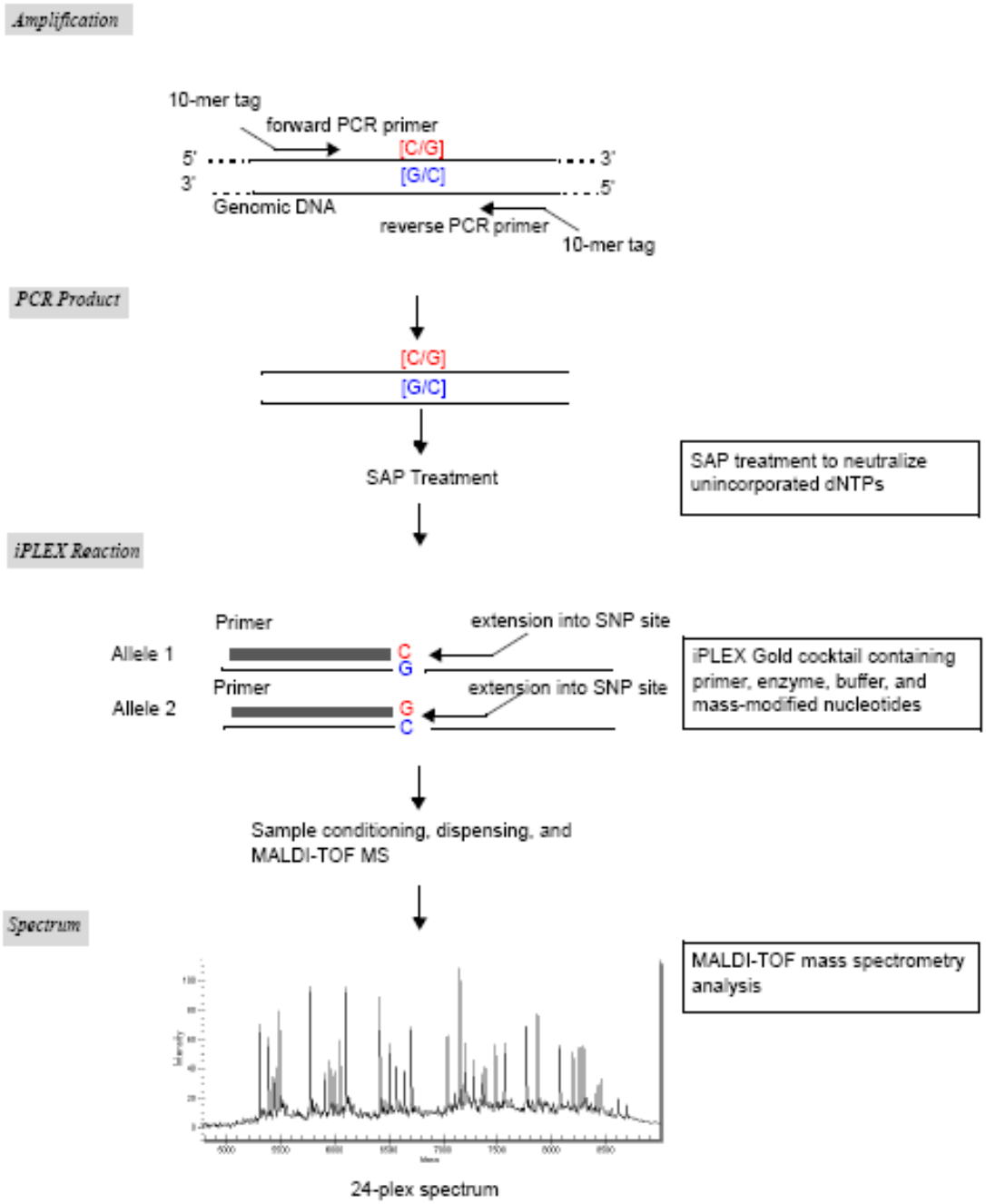

Fig. 5.: Schematic of iPLEX reaction

Depending on the present allele, products differing in length and mass are generated. These differences are subsequently shown by MALDI TOF mass spectrometry

(SEQUENOM, iPLEX Gold ApplicationGuide, November 2006).

2.10 SNP Detection by Means of Matrix-assisted Laser Desorption Ionisation (MALDI) Time of Flight (TOF) Mass Spectrometry (MS)

MALDI TOF MS is an analytical method for determination of molecular masses of free ions in high vacuum. The MALDI TOF MS was developed 1987 by Hillenkamp und Karas. Formerly, this method was for mass determination of bigger molecules (such as peptides and proteins). Nowadays it is also used in SNP analysis.

For sample preparation different techniques were developed, all guaranteeing intercalation of the analyte molecule into the lattice of the matrix (Karas and Hillenkamp 1988); (Kirpekar, Nordhoff et al. 1998). A common matrix is 3-hydroxy-picolin-acid. This is an aromatic, carboxylcontaining acid, giving its proton to the negative oligonucleotides of the sample in a lattice structure and therefore allowing ionisation. Matrix substance absorbs radiated laser energy and protects, due to the 100- to 1000-times overplus, the analyte molecules from its degradation. Moreover the matrix is to avoid damaging of the analyte and interaction of analyte molecules among each other and between analyte and sample carrier (Hillenkamp, Karas et al. 1991). Analyte and matrix build a so-called MALDI-plum.

During MALDI TOF MS the sample is irradiated few nanoseconds with short waved laser light. This yields a local breakup of the solid surface. The absorbed energy is passed down to the samples molecule, imbedded in the matrix. Through this the sample molecule is desorbed, ionized and vaporized. The whole process is carried out in a vacuum (Gut 2001); (Hillenkamp, Karas et al. 1991).

A negative electrode, located next to the sample, generates an electrostatic field. Positive sample ions are accelerated from the sample surface towards the analyzer. For MALDI-analyses a time of flight spectrometer is used. Mass determination is accomplished by exact measurement of elapsed time between start of ion from the sample until arrival at the detector. Ions with smaller masses and equal kinetic energy can be accelerated more than heavier ions. At constant acceleration voltage and flight distance the measured time of flight correlates with a certain mass (Griffin and Smith 2000).

Calibration is accomplished with a reference substance of known mass. Typical flight times at the MALDI TOF MS are around 100 microseconds and drift distance is 1 – 4 m (Fig. 6).

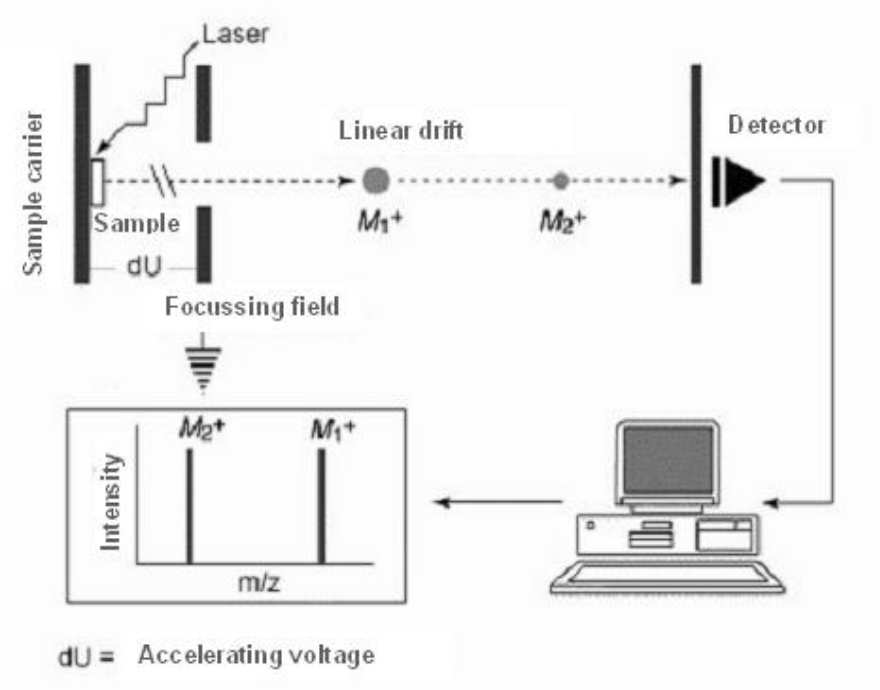

Fig. 6.: Schematic of MALDI TOF mass spectrometry according to Griffin TJ et al.

Vaporization of matrix by means of the laser the oligonucleotide is accelerated and moves in the field-free flight tube with mass- and chargedependent velocity towards the detector. Here dissociation and detection of two oligonucleotides with different masses/charges are described.

Thanks to the development of MALDI TOF mass spectrometry it is now possible, to determine DNA fragments in the range of 1000 to 9000 Da, i.g. 3 – 30 bases with an accuracy of 0.1 to 0.01 %. Therefore a mass range is opened up, where SNP analysis via primer extension products can be performed. Adequate reaction conditions and primers were calculated by the SpectroDesigner Software (see § 3.2.3) and the respective allele of the selected SNPs were analyzed by means of MALDI TOF MS (Fig. 7).

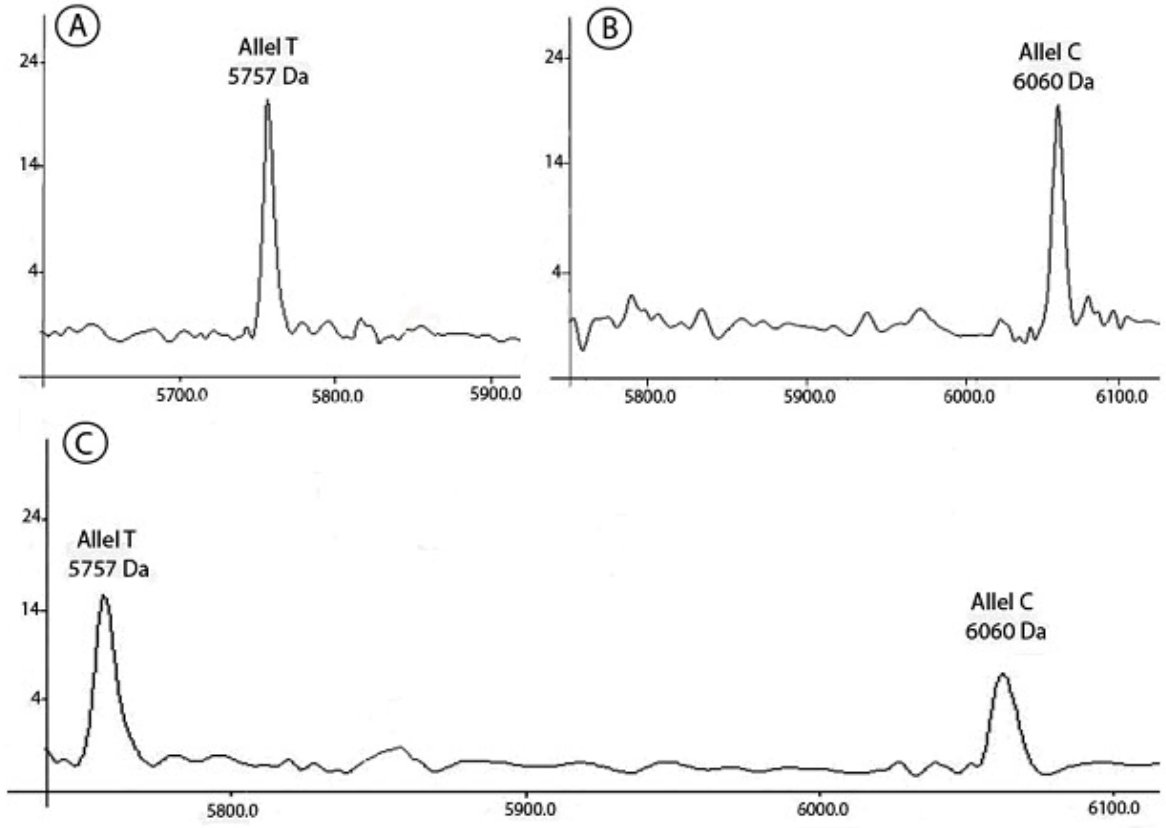

Fig. 7.: MALDI TOF MS analysis of the primer extension reaction product

MALDI TOF MS analysis of the primer extension reaction product of an example SNP [C/T]. A: shows the homozygot condition of the allele T, B: shows homozygot condition of the allele C and C: shows the heterozygot condition. (X axis – mass, Y axis – intensity)

Genotyping was performed simultaneously for several SNPs (multiplex reaction) in a 384-well format according to an optimized protocol. Primers were designed by the SpectroDesigner Software such that the received products can be divided by mass (Fig. 8.). Therewith, overlaps of different allelespecific products with the same mass were avoided. Another problem is the possible weak signal intensity of individual products.

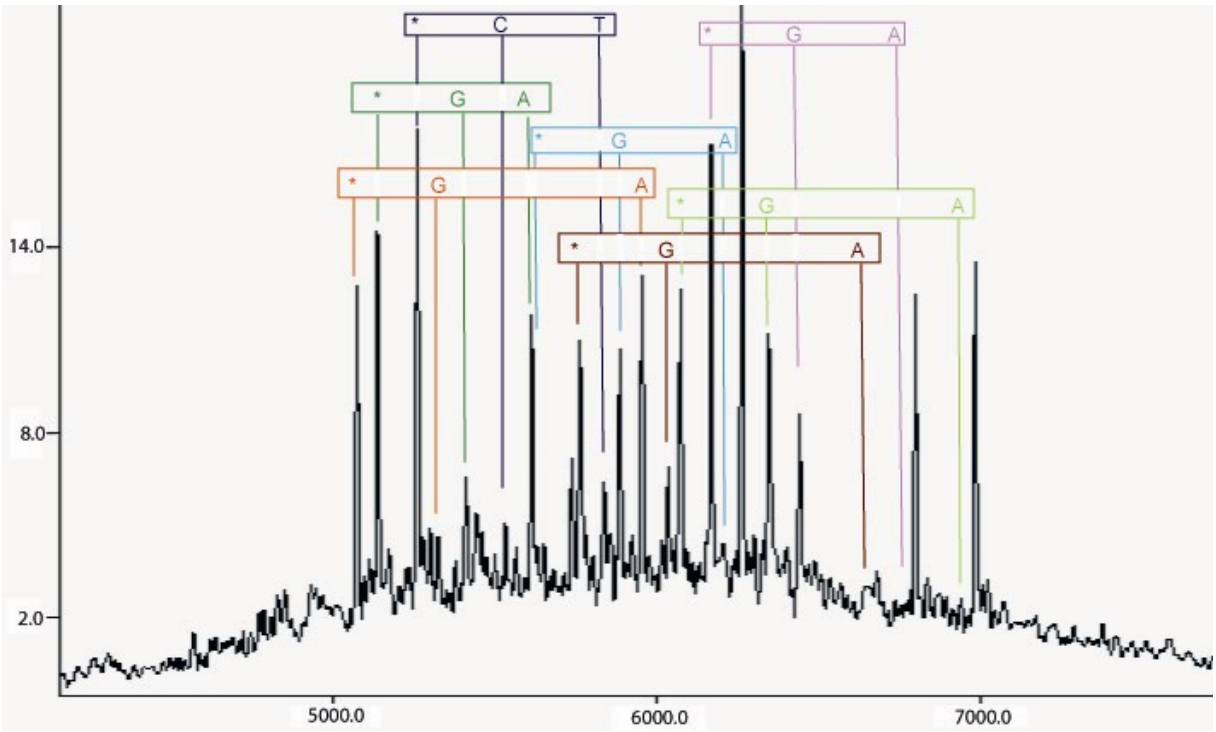

Fig. 8.: MALDI TOF MS analysis of a multiplex reaction

MALDI TOF MS analyis of a multiplex reaction of seven genotyped biallelic SNPs: The mass spectrum shows the analysis of the primer extension reaction for seven example SNPs, example SNP 1 [G/A] (marked red), example SNP 2 [G/A] (marked in green), example SNP 3 [C/T] (marked in blue), example SNP 4 [G/A] (marked in light blue), example SNP 5 [G/A] (marked in dark red), example SNP 6 [G/A] (marked in light green) und example SNP 7 [G/A] (marked in pink). Asterisks show the mass region, where the respective primers without extension were detected.

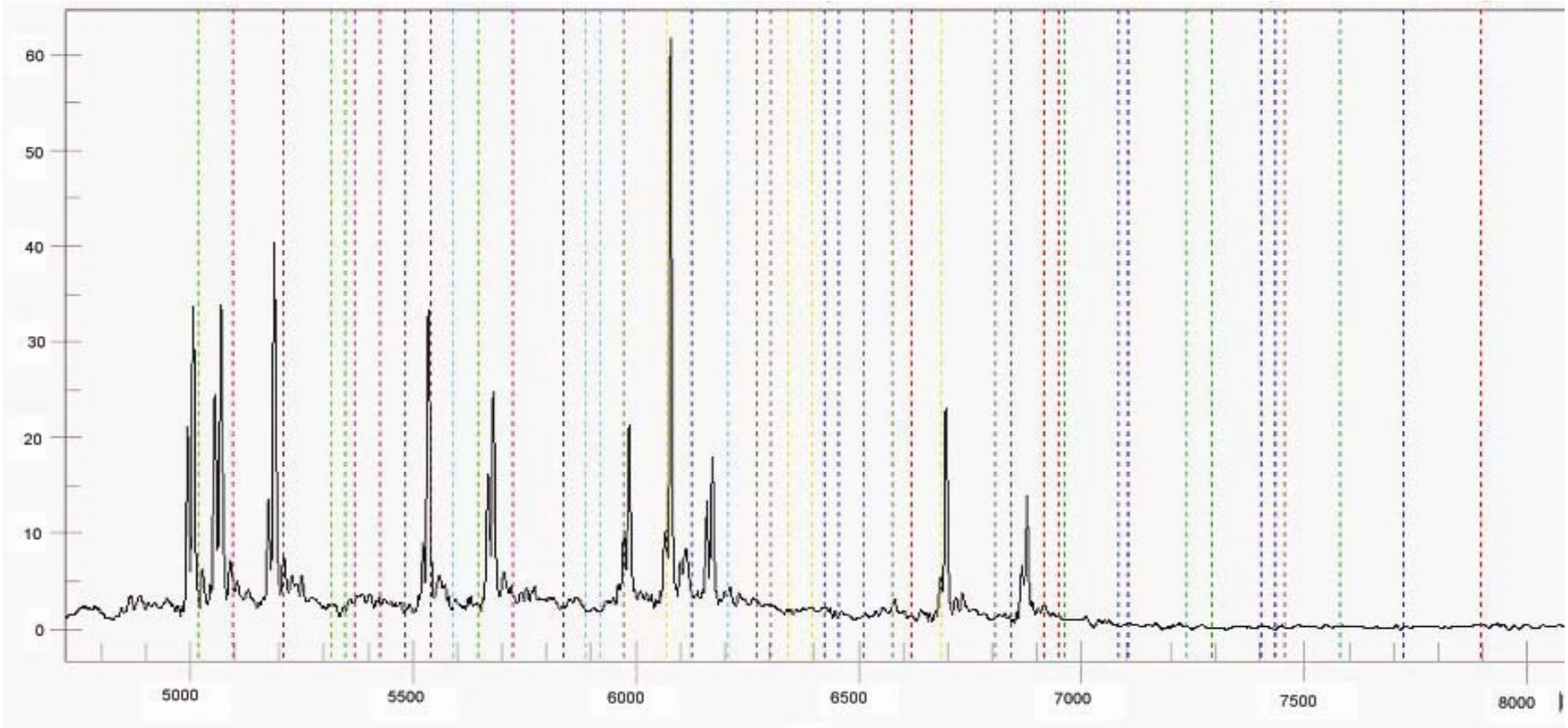

Fig. 9.: MALDI TOF MS analyses of extension primers for *APP*

MALDI TOF MS analyses of example extension primers for APP. The x-axis presents the mass, the y-axis the intensity of the signal. Peaks for the same SNP are marked in the same colours.

2.11 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Using RT-PCR (reverse transcription polymerase chain reaction) the amount and type of gene transcription and therewith the amount and type of gene expression can be determined.

RNA – isolated as described in § 2.3.2 from brain samples – is specifically reverse transcribed. Control RNA of defined concentration (Applied Biosystems FirstChoice® Human Brain Reference RNA, 1 mg/mL, Part No. AM6050) was treated the same way, for calibration and checking of the procedure. The used enzyme, reverse transcriptase (RT) is a RNA-dependent DNA-polymerase. It allows efficient synthesis of first strand cDNA from RNA templates. For initiation of single stranded cDNA synthesis poly-(dT) primers are used. They allign to the poly-A-sequence and are used for transcription of mRNA, while rRNA - due to the lack of poly-A sequence - can not be reverse transcribed (Ando, Monroe et al. 1997; Malhotra, Foltz et al. 1998).

A commercial cDNA synthesis kit was used (RevertAidTM H Minus First Strand cDNA Synthesis Kit #K1631, Fermentas GmbH, 68789 St. Leon-Rot, Germany).

Tab. 7: Pipetting scheme cDNA synthesis

Volume:	Substance	Concentration
5 µL	RNA	1-2 µg per approach
1 µL	Poly-(dT) Primer	0.5 µg/µL
4 µL	5x reaction buffer	250mM Tris-Hcl, 250mM MgCl2, 50mM DTT
0.5 µL	RNase Inhibitor	20 U/µL
2 µL	dNTP-Mix	10mM each dNTP
1 µL	RevertAid™ H Minus M-MuLV Reverse	200 U/µL

Tab. 8: Conditions for cDNA synthesis

Time:	Temperature:	Step:
5 min	70°C	Denaturation
5 min	37°C	Incubation
60 min	42°C	Reverse transcription
10 min	70°C	Inactivation of enzyme

Subsequently, the obtained cDNA was amplified via RTQ-PCR (real time quantitative PCR, see below).

2.12 Real Time Quantitative Polymerase Chain Reaction (RTQ-PCR) - Gene Expression Analysis

RTQ-PCR (real time quantitative PCR) is based on the principals of common PCR protocols (see above), but additionally allows a quantification of the obtained DNA. Quantification is accomplished by means of fluorescence measurements, which are recorded during each PCR cycle. Fluorescence signals increase proportional with the amount of RTQ-PCR products.

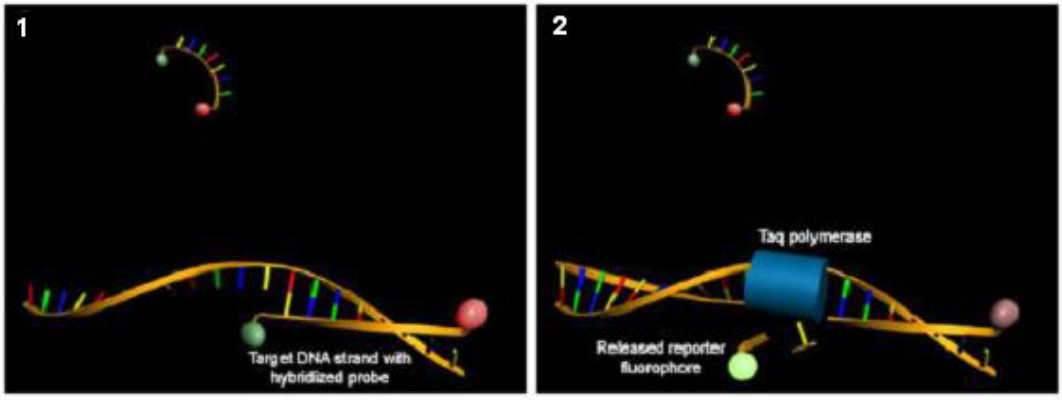

Fig. 10.: Schematic of RTQ-PCR

(1) Probes and c DNA strand are hybridized and reporter fluorescence is still quenched. (2) During PCR, the probe is degraded by the Taq polymerase and the fluorescent reporter released

(Pfaffl 2004)

Gene expression analysis was carried out using ready made TaqMan® Gene Expression Assays (APP, Hs00169098_m1; ABCA1, Hs00194045_m1; 18S ribosomal RNA (endogenous control), Hs99999901_s1), Applied Biosystems, Foster City, California USA and TaqMan® Universal PCR Master Mix, NoAmpErase® UNG manufactured by Roche, Applied Biosystems, Branchburg, New Jersey USA) on a TaqMan 7300 system (Applied Biosystems, Foster City, California USA) as per the manufacturers instructions. Alignment maps for TaqMan® Gene Expression Assays of APP and ABCA1 are depicted in Fig. 11 and Fig. 12 below. Arbitrary units (AU) of gene expression were determined using the method described by Barrachina and colleagues (Barrachina, Maes et al. 2006).

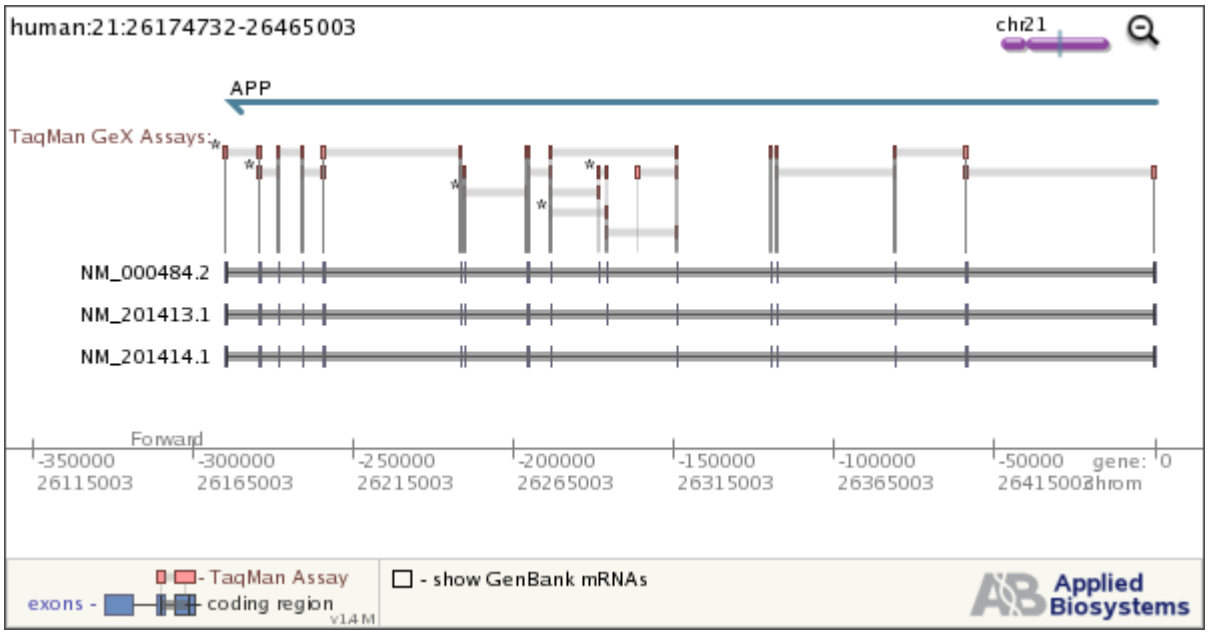

Fig. 11.: **Alignment map for TaqMan® gene expression assay for APP**

(https://products.appliedbiosystems.com)

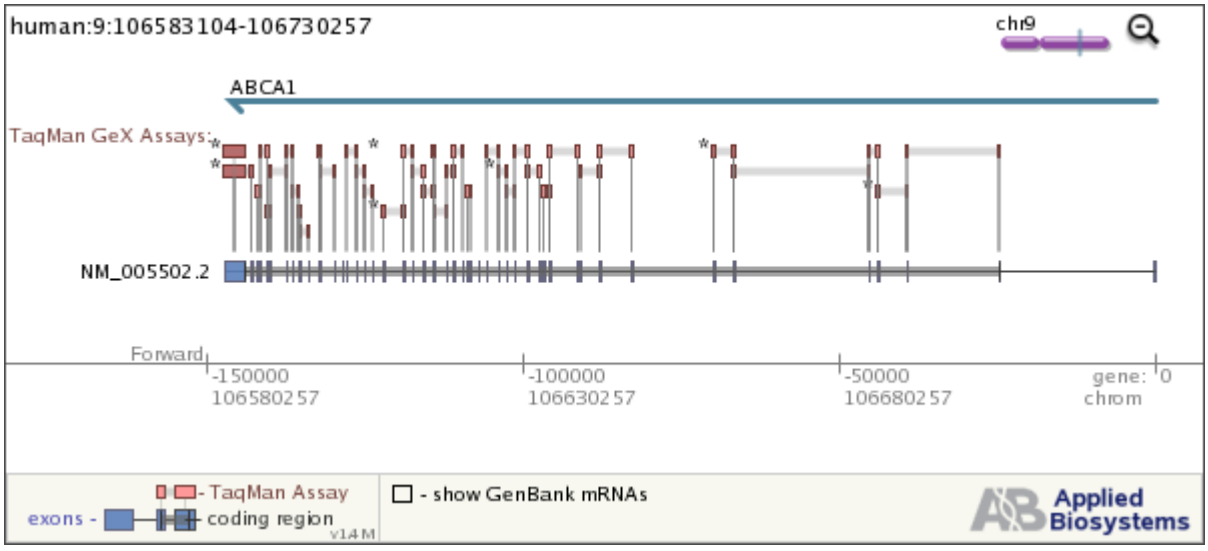

Fig. 12.: **Alignment map for TaqMan® gene expression assay for ABCA1**

(https://products.appliedbiosystems.com)

Tab. 9: Pipetting scheme RTQ-PCR

Volume:	Substance	Concentration
9 µL	cDNA	corresponding to cDNA from approx. 4 ng of RNA
1 µL	20×TaqMan® Gene Expression Assays	
10 µL	2×TaqMan Universal PCR Master Mix	

Tab. 10: Conditions for RTQ-PCR

Time:	Temperature:	Step:
10 min	95°C	Initial denaturation
15 sec	95°C	Denaturation
1 min	60°C	Primer annealing and extension

2.13 Measurement of Different Aβ isoforms

Measurement of different Aβ isoforms (Aβ38, Aβ40 and Aβ42) in total human brain homogenates was carried out with a commercial multi-plex ELISA kit (MSD® Human (6E10) Aβ 3-Plex 38/40/42 Kit, MULTI-SPOT®4, 96-well Plate, Merck & Co., Inc., whitehouse station, NJ, USA) as per the manufacturer's instructions. An ELISA (enzyme-linked immunosorbent assay) is an immunological assay on basis of an enzymatic fluorescence reaction.

Total brain homogenates that were also used in the above assay were prepared in PBS, containing a protease inhibitor cocktail (5 µg/mL aprotinin, 5 µg/mL leupeptin, and 0.1 mM phenylmethylsulfonyl fluoride, all from Sigma, St. Louis, MO, USA), at a volume of 3 ml/g of tissue and homogenised for 15 seconds. The concentration of total protein in the tissue homogenate was determined using the micro-bicinchoninic acid protein assay kit (Pierce).

Aβ42 levels were determined in CSF (cerebrospinal fluid), prepared as described above, using a commercial ELISA kit (INNOTESTTM ß-Amyloid(1-42), INNOGENETICS®, Gent, Belgium) as described in greater detail elsewhere (Vanderstichele, Van Kerschaver et al. 2000). Plasma Aβ42 levels were determined using the high-sensitivity protocol of the above ELISA kit.

2.14 Western Blot

A western blot approach was optimized and carried out for total brain homogenates as described below. The used primary antibody, WO2, is a monoclonal antibody, which recognises the Aβ peptide and therefore unprocessed APP as well as β-secretase processed APP (CTFβ).

Total protein amount of each brain sample was determined and thereafter, brain samples were diluted with gel loading buffer, so that 25 µg total protein were achieved. Thereafter, accordingly diluted samples (brain homogenates, standard or marker) were quick vortexed, quick centrifuged, boiled 5 min at 100°C, quick centrifuged and loaded onto a 10 – 15 % SDS PAGE (sodium dodecylsulfate polyacrylamide gel electrophoresis) and processed at constant V (60mA per gel). Contained APP and Aβ is divided on the gel and form two bands, later on visible on the gel after performance of the western blot. The gel was transferred onto a preconditioned membrane (10 sec. in MeOH, 1-2 min in H2O, 10 sec in Transferbuffer for equilibration) over night in a TransBlotCell at 4°C and constant V, 250 mA). Each membrane was then cut horizontally at different molecular weight marker levels to use different sections of the same membrane to probe for proteins with differing molecular weights. The next day, the membrane was treated as follows:

- cooked in 1x PBS for 5 min
- cooling down
- blocked for 45 min in casein buffer
- incubated for 2 h in the primary antibody (W02 1:2000 in TBST)
- washed 4 times for 10 min in 1x TBST
- incubated for 1 h in the secondary antibody (1:4000 anti mouse IgG in TBST) -
- washed 4 times for 10 min in 1x TBST
- incubated for 5 min in substrate (ECL Plus Western Blotting Reagent Pack)

If not otherwise noted, steps were carried out at room temperature under slow shaking.

Tab. 11: Buffers used for Western Blot

Loading buffer for gel	900 µL SDS buffer 100 µL β-MercaptoMeOH
Transfer buffer	200 mL 10x Transfer 1400 mL dd H_2O 400 mL MeOH
10x TBS	200mM TrisHCl 1.5 M NaCl
1x TBST	100 mL 10x TBS Stock 0.05% (v/v) Tween 20 (500 µL) ad 1L dd H_2O
Casein buffer	2.5 g Casein 50 mL 1M NaOH 950 mL dd H_2O

The recorded picture of the membrane was thereafter scanned and safed as a *.tif-file. Density was evaluated from this picture with ImageJ (a public domain, Java-based image processing program developed at the National Institutes of Health). Densities of the different bands (APP due to its higher mass the upper band in each lane and Aβ the lower band) were measured as "Integrated densities" by the program. Background correction was performed by subtraction of band-free gel background from each value.

2.15 Isolation of Cerebrospinal Fluid

Isolation of cerebrospinal fluid (CSF) was performed by physicians as described in the following paragraph. Spinal taps and venous punctures had previously been performed in these individuals after written consent had been obtained. CSF (5-8 mL) was collected in sterile polypropylene tubes using atraumatic canules placed in the intervertebral space L3/L4 or L4/L5. Serum and EDTA plasma were obtained by venous puncture. In native CSF routine chemical parameters were within normal range in all subjects. These parameters included leukocyte and erythrocyte cell count as well as glucose and lactate measurement, total protein content, CSF to serum ratio of albumin and immunoglobulin G, and a screening for oligoclonal bands. CSF was centrifuged for 10 minutes at 4000 g. The remaining CSF supernatants were immediately frozen, in aliquots, at -80°C for subsequent analysis of Aβ42 levels.

2.16 Statistical analyses

2.16.1 Hardy-Weinberg-Equilibrium (HWE)

For eradication of genotyping errors, the control group was tested for each SNP with the χ^2 Test for deviations from the Hardy-Weinberg-Equilibrium (HWE). In the subsequent statistical analysis only those SNPs were taken into account, whose genotypic distribution showed no significant deviation from the HWE as set in the default settings of the Haploview software. Test for HWE was only performed for SNPs with sufficient genotyping efficacy (≥ 95%).

2.16.2 Allelic und Genotypic Test

Association between allele or genotype distribution of each SNP and the AD phenotype was tested.

Allelic and haplotypic associations were tested for significance using χ2-tests, as implemented in Haploview version 4.2 (Barrett, Fry et al. 2005). Genotypic associations with AD were tested by logistic regression analyses. For that gender and age at onset (for cases)/age (for controls) were included as covariates. For evaluation of pooled collectives population was additional included as covariate. Logistic regression analyses, using recessive trend model, were undertaken in the Statistical Package for Social Sciences (SPSS version 16; SPSS Inc., Chicago, Illinois, USA) or in STATA SE v9.2 (StataCorp, College Station, Texas, USA).

Association analyses for the sib-pairs cohort was undertaken using Family Based Association Testing software (FBAT version 2.0.2C) (Laird, Horvath et al. 2000). Genotyping results from the sib-pairs cohort were analyzed for association with AD by means of Sib Transmission / Disequlibrium Test (S-TDT) (Spielman and Ewens 1998)). S-TDT is a modified version of the Transmission / Disequlibrium Test (TDT) (Spielman, McGinnis et al. 1993). The TDT checks, whether heterozygote parents inherit an allele, which is associated with the disease, to their diseased children significantly more frequent, than according to Mendel´s laws (if no linkage or association exist). Late onset diseases have proven to be particularly problematic in terms of missing information about the parents. In this case the S-TDT was applied. It applies reconstruction of parental alleles through the data of siblings.

2.16.3 Haplotype Analyses and Linkage Disequilibrium Structure

As a measure of linkage disequilibrium (LD) between each possible pair of SNP loci, Lewontin's disequilibrium coefficient D' and the coefficient of determination r^2 (squared correlation coefficient) were estimated. Haplotype block definition was determined via the confidence interval approach of Gabriel et al., 2002 (Gabriel, Schaffner et al. 2002), all as implemented in Haploview version 4.2 (Barrett, Fry et al. 2005). Haplotype estimations and haplotype analyses were performed by EM algorithms implemented in Haploview (Barrett, Fry et al. 2005). Via Haploview frequencies of haplotype combinations were evaluated and their association with AD was tested.

2.16.4 Sliding Window Analysis of 2- and 3-marker Haplotypes

Sliding window analyses were carried out using the software package Haploview version 4.2. Frequencies for each haplotype combination of 2 or 3 adjacent SNPs were calculated and an association test was performed subsequently. Haplotype combinations with frequencies below 5% were not taken into account, since their occurrence seems to be very unlikely.

A graphical sliding window approach of plotted p-values of 2 and 3-marker haplotypes as described above was thereafter conducted in Microsoft Excel 2003 (Microsoft Corp., Redmond, USA).

2.16.5 Statistical Analysis of Functional Tests

Genotypic associations with results from functional analysis (gene expression, analysis of different Aβ isoforms and Aβ42 levels) were undertaken for selected SNPs and were performed using The Statistical Package for Social Sciences (SPSS version 16; SPSS Inc., Chicago, Illinois, USA) using t-test or ANOVA (analysis of variance). Results from western blot analyses were evaluated with ANOVA. Multiple testing using Tukey LSD = Least Significant Difference and HSD = Honest Significant Difference was used for ANOVA also as implemented in SPSS. For parametric tests, normally distribution was tested by means of the Kolmogorov-Smirnov-Test as implemented in SPSS, as well.

Further, association of SNPs with clinical data were investigated using linear regression models, controlling for duration and gender (MMSE scores) or gender (age at onset), and were likewise performed within SPSS.

3 Materials

3.1 Laboratory Equipment and Solutions

3.1.1 Laboratory Equipment

In the scope of this study, laboratory equipment as specified in Tab. 12 was used.

Tab. 12: List of materials and providers – part 1

Materials	Type and Provider
Pipets	Eppendorf Pipets (Eppendorf AG, Hamburg, Germany) Gilson Pipets (Gilson Company Inc, Lewis Center, USA) Brand Pipets (Brand GmbH + Co KG, Wertheim, Germany)
Centrifuge	Centrifuge 5415 D (Eppendorf AG, Hamburg, Germany)
Vortexer	MS2 Minishaker (IKA-Works, Wilmington, USA)
Gel electrophoresis apperature	Power Pack PL (Biometra GmbH, Göttingen, Germany) Power Pac 3000 (Bio-Rad Lab. GmbH, München, Germany) Sunrise™, horizontal Gel Electrophoresis Apparatus (GibcoBRL, Life Technologies, Karlsruhe, Germany) Sub cell Model 192 (Bio-Rad Lab. GmbH, München, Germany)
Gel documentation system	UVT-40M Transilluminator (Herolab, Wiesloch, Germany) Mighty Bright UVTM Transilluminator (Hoefer Inc., San Francisco, CA, USA) E.A.S.Y 429 K Kamera (Herolab, Wiesloch, Germany) Hoefer's PhotoMan (Hoefer Inc., CA, USA)
Centrifuge (for plates)	Sigma 4K15C (Sigma laborzentrifugen, Osterode, Germany) Rotanda 46RS (Hettich, Darmstadt, Germany) Zentrifuge 5810 R (Eppendorf AG, Hamburg, Germany)
Microwave	Siemens 32L (Siemens, München, Germany)
Photometer	Ultrospec 3100 pro (Biochrom Ltd., Cambridge, UK) Smart Spec™ (Bio-Rad Lab. GmbH, München, Germany)
Ultrapure Water Purification System	UltraClear UV (SG Wasseraufbereitung und Regenerisation GmbH, Barsbüttel, Germany) Millipore RiOs™ 8 (Millipore, Billerica, Mass., USA)
Mass spectrometer	Autoflex (Bruker Franzen Analytik GmbH, Bremen, Germany)
Thermomixer	Thermomixer comfort (Eppendorf AG, Hamburg, Germany) BT 130-2 (HLC Haep Labor Consult, Bovenden, Germany)
Spotter	SpectroPoint™ Nanoliter Pipetting System (SEQUENOM GmbH, San Diego, CA, USA)
PCR Cycler	PCR PTC 225 Tetrad, Peltier Thermal Cycler (MJ-Research, Boston, USA) Gene Amp™ PCR System 9700 (Applied Biosystems, Foster City, CA, USA) Primus 96 Plus (MWG-Biotech AG, Ebersberg, Germany)

Pipetting robot	Genesis RSP 150 Work Station (Tecan AG, Crailsheim, Germany) Multimek 96 Automated 96-Channel Pipettor (Beckman Coulter, Fullerton, USA) Temo (Tecan AG, Crailsheim, Germany) Miniprep 75 (Tecan AG, Crailsheim, Germany)
Balance	BL 610 (Sartorius AG, Göttingen, Germany) BP 121 S (Sartorius AG, Göttingen, Germany)
Ice machine	AF 30 (Scotsman, Mailand, Italy)
Shaker	Taumelschüttler Unitwist 3D (UniEquip Laborgeräte + Vertriebs GmbH, München, Germany) ROTO-Shake Genie (Scientific Industries, Inc Bohemia, USA) Rocky (Fröbel GmbH, Lindau, Germany)
Homogenizer	Ultra-Turrax T8 (IKA-Werke GmbH & Co. KG, Staufen, Germany) T8.01 Netzgerät (IKA-Werke GmbH & Co. KG, Staufen, Germany)
TransBlot Cell	Trans-Blot® Electrophoretic Transfer Cell (Bio-Rad Laboratories GmbH, München, Germany)

3.1.2 Reagents, Solutions, Buffers and Kits

Materials, their provider, as well as their usage are listed in the table below:

Tab. 13: List of materials and providers – part 2

Materials	**Methods**	**Provider**
Puregene DNA Isolation Kit	DNA Isolation	Gentra, Mineapolis, USA
TE Puffer [(Hydroxylmethyl)Aminomethan-Ethylendiaminotetraacetyl-Säure]	DNA Verdünnung	Gentra, Mineapolis, USA
Isopropanol	DNA and RNA Isolation	Sigma-Aldrich, Osterode, Germany
Ammoniumacetat	DNA Isolation	Pharmacy of TUM
RNeasy Lipid Tissue Mini Kit	RNA Isolation	Qiagen GmbH, Hilden, Germany
10x PCR Puffer	PCR	Qiagen GmbH, Hilden, Germany
25mM MgCl2	PCR	Qiagen GmbH, Hilden, Germany
dNTPs	PCR	Paqlab, Erlangen, Germany
Agarose Ultra Pure	Gelelectrophoresis	Paqlab, Erlangen, Germany
TBE running buffer (Tris-Borat-buffer)	Gelelectrophoresis	Merck KGaA, Darmstadt, Germany

Ethidiumbromid	Gelelectrophoresis	Bio-Rad Lab. GmbH, München, Germany
DNA loading buffer for agarose gels	Gelelectrophoresis	Bio-Rad Lab. GmbH, München, Germany
Marker for DNA agarose gels	Gelelectrophoresis	Bio-Rad Lab. GmbH, München, Germany
RNA loading buffer for agarose gels	Gelelectrophoresis	Sigma-Aldrich, Osterode, Germany
MOPS Buffer	Gelelectrophoresis	Sigma-Aldrich, Osterode, Germany
Formaldehyd	Gelelectrophoresis	Sigma-Aldrich, Osterode, Germany
iPLEX Buffer	iPLEX	Sequenom GmbH, San Diego, CA, USA
iPLEX termination mix	iPLEX	Sequenom GmbH, San Diego, CA, USA
iPLEX enzyme	iPLEX	Sequenom GmbH, San Diego, CA, USA
Spectro CLEAN kit	Cleaning up of iPLEX products	Sequenom GmbH, San Diego, CA, USA
Calibrant	Spotting	Sequenom GmbH, San Diego, CA, USA
Acetic acid		Merck KGaA, Darmstadt, Germany
Ethanol		Merck KGaA, Darmstadt, Germany
Sodium hypochlorite		Sigma-Aldrich, Osterode, Germany
Sodium hydroxid		Merck KGaA, Darmstadt, Germany
Sodium acetate		Merck KGaA, Darmstadt, Germany
Ethylenediamine tetraacetic acid (EDTA)		Merck KGaA, Darmstadt, Germany
RiboLock™ RNase Inhibitor	RT-PCR	Fermentas GmbH, St. Leon-Rot, Germany
Applied Biosystems FirstChoice® Human Brain Reference RNA, 1 mg/mL, Part No. AM6050	RT-PCR	Applied Biosystems, Foster City, California USA
TaqMan® Gene Expression Assays (Hs00169098_m1, Hs99999901_s1 Hs00194045_m1	RTQ-PCR	Applied Biosystems, Foster City, California USA
TaqMan® Universal PCR Master Mix	RTQ-PCR	Roche, Applied Biosystems, Branchburg, New Jersey USA
NoAmpErase® UNG	RTQ-PCR	Roche, Applied Biosystems, Branchburg, New Jersey USA
MSD® Human (6E10) Aβ 3-Plex 38/40/42 Kit, MULTI-SPOT®4, 96-well Plate,	Aβ isoform determination	Merck & Co., Inc., whitehouse station, NJ, USA
INNOTEST™ ß-Amyloid(1-42)	Aβ 42 determination	INNOGENETICS®, Gent, Belgium

ECL Plus Western Blotting Reagent Pack	Western blot	GE Healthcare, Amersham, UK
Amersham Hybond™-P	Western blot	GE Healthcare, Amersham, UK
Acryl amide	Western blot	Bio-Rad Laboratories GmbH, München, Germany
$W0_2$ (primary antibody)	Western blot	Gift from Tobias Hartmann
Anti mouse IgG HRP linked antibody	Western blot	Sigma-Aldrich, Osterode, Germany Munich, Germany
Casein	Western blot	Sigma-Aldrich, Osterode, Germany
β-mercaptoMeOH	Western blot	Sigma-Aldrich, Osterode, Germany
Tris HCL	Western blot	Sigma-Aldrich, Osterode, Germany
Tween 20	Western blot	Bio-Rad Laboratories GmbH, München, Germany
NaOH	Western blot	Sigma-Aldrich, Osterode, Germany
NaCl	Western blot	Sigma-Aldrich, Osterode, Germany
Rainbowmarker	Western blot	GE Healthcare, München, Germany

3.1.3 Enzymes

Used enzymes in the present work, as well as their providers are reported in the table below:

Tab. 14: List of enzymes and providers

Enzyme	Provider
HotStar Taq DNA Polymerase	Qiagen GmbH, Hilden, Germany
Shrimp Alkaline Phosphatase (SAP)	Sequenom GmbH, San Diego, CA, USA
Thermosequenase	Sequenom GmbH, San Diego, CA, USA
RevertAid™ H Minus M-MuLV Reverse	Fermentas GmbH, St. Leon-Rot, Germany

3.1.4. Oligo-nucleotides

PCR primers were synthesized by Metabion GmbH (Martinsried, Germany), Invitrogen (Karlsruhe, Germany) and MWG-Biotech AG (Ebersberg, Germany). They were HPLC-purified and MALDI TOF MS documented. All PCR-primers, which were used for MALDI TOF MS, were tagged with hME-Tag (5´-ACGTTGGATG-3`). A list of all used primers is shown in the Appendix (see § 7.5.1).

3.2 Software

3.2.1 Software for Pipetting Robots

- Gemini 3.5 (Visual Basic Programme), Tecan, Crailsheim, D
- Bioworks, Beckmann Coulter, Fullerton, USA

3.2.2 MALDI TOF MS Software

- SpectroTyper RT™, Sequenom GmbH, San Diego, CA, USA
- MassARRAY Typer, Sequenom GmbH, San Diego, CA, USA
- SpectroREADER, Sequenom GmbH, San Diego, CA, USA
- Pintool Software, Sequenom GmbH, San Diego, CA, USA, RoboDesign USA
- SpectroDesigner™ 2.1, Sequenom GmbH, San Diego, CA, USA
- SpectroImporter Sequenom GmbH, San Diego, CA, USA

3.2.3 Software for Primer Design and Determination of PCR Conditions

- VectorNTI Suite 6.0, VectorNTI Suite 7.0, VectorNTI Advance 9.0 and VectorNTI Advance 10.3
- SpectroDesigner™ , Sequenom GmbH, San Diego, CA, USA
- FastPCR © v. 4.0
- NCBI BLAST (http://130.14.29.110/BLAST/)
- Human BLAT Search (http://genome.ucsc.edu/cgi-bin/hgBlat)

3.2.4 Software for Analyses of Genomic DNA Sequences

- VectorNTI Suite 6.0, VectorNTI Suite 7.0, VectorNTI Advance 9.0 and VectorNTI Advance 10.3

3.2.5 Software for Evaluation of Western Blot

- ImageJ (http://rsb.info.nih.gov/ij/developer/index.html)

3.2.6 Databanks

- National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov/)
- ensEMBL (http://www.ensembl.org)
- CHIP Bioinformatics Tools (http://www.snpper.chip.org/)
- Celera (http://www.celeradiscoverysytem.com/)
- HapMap (http://www.hapmap.org/)
- NeoRef TAMAL (http://neoref.ils.unc.edu/tamal/)
- UCSC Genome Bioinformatics (http://www.genome.ucsc.edu/)
- Gene Regulation Biobase (http://www.gene-regulation.com/)
- Genomatix (http://www.genomatix.com/)
- NEBcutter V2.0 (http://tools.neb.com/NEBcutter2/)

- ESEfinder (http://rulai.cshl.edu/tools/ESE/)
- European Bioinformatics Institute (http://www.ebi.ac.uk/)
- Alzheimer Research Forum (http://www.alzforum.org/)

3.2.7 Statistical Programs

- Statistical Package for the Social Sciences (SPSS), Version 16.0
- Haploview, Version 4.1
- STATA SE 9.2 for Windows

4 Subjects

4.1 Criteria of Inclusion

Subjects investigated in this work were recruited and phenotyped in university hospitals in Germany and Sweden.

The following criteria of inclusion were taken into account during selection of subjects:

1. Due to ethical and legal reasons an informed consent in written form has to be available for each subject. For affected subjects their persons in charge had to sign.
2. Only ethnic Germans were incorporated into the Munich collective of subjects. Thus, possible ethnic differences of genotype and phenotype are controlled for each study design.
3. Phenotyping was carried out on basis of the criteria as described in chapter 2.1.

4.2 Selection and Composition of Subjects for Alzheimer Studies

4.2.1 Initial Case-Control Study from Munich

The subjects for the Munich cohort (MUC) were recruited at „Klinik für Psychiatrie und Psychotherapie der TUM, Klinikum rechts der Isar“. Diagnoses were made according to the above mentioned criteria.

The phenotypic association main study consisted of a total of 728 Caucasian subjects comprising 438 individuals with a diagnosis of probable AD (mean age, 72.2 years ± standard deviation 9.1; age at onset, 69.0 years ± 9.1; 41% males) and 290 cognitively healthy controls (mean age, 66.8 years ± 12.0; 44% males). Sub-collectives were composed of the main case–control cohort described above. For this purpose the main collective was divided by age of onset (sub-collective *EOAD*: onset ≤ 65 years, *LOAD*: onset > 65 years). Additional stratification by the presence of at least one copy of the *ApoE* ε4 allele was also performed in

the Munich cohort. A detailed table on the composition of the whole collective and the sub-cohorts is shown in Tab. 45.

Individuals from the main patient series were recruited from a university memory clinic from 1994 to 2004. The control group was matched for geographical location and ethnicity and consisted of cognitively healthy subjects who were recruited from spouses of those attending the memory clinics and community based geriatric daycare units. Information on age at onset of the disease was obtained from an informant. Age at onset was defined by the appearance of first clinical symptoms. After informed consent had been obtained, blood samples of each subject were taken by venous puncture. The study protocol was approved by the review board of the medical faculty, Technische Universität München. The clinical diagnosis of probable AD was established according to the NINCDS-ADRDA criteria. Cognitive impairment was assessed using the Cambridge Cognitive Examination or, alternatively, the Consortium to Establish a Registry for Alzheimer's disease. Both instruments incorporate the Mini Mental State Examination, which was used to test cognitive performance in the control group. Control subjects with a MMSE score below were excluded from further analysis. All patients and controls underwent a thorough psychiatric, neurological and neuropsychological evaluation. The diagnostic workup also included an informant interview, a chemistry survey, structural magnetic resonance imaging (MRI) imaging and functional imaging in patients with AD whenever possible.

4.2.2 Case-Control Studies for Replication

Sigificant association results as identified in the Munich collective (MUC) were replicated in an additional case-control study. For replictation of the results subjects from Sweden (Stockholm) were genotyped and statistically evaluated.

In collaboration with the Karolinska institute in Stockholm (Sweden) a replication study analysis consisting of a total of 883 Swedish subjects comprising 252 individuals with a diagnosis of probable AD (mean age and onset 67.9 years, standard deviation (s.d.) 6.1; 40% male) and 631 cognitively healthy controls (mean age, 73.1 years s.d. 8.9; 39.4% male).

A detailed table on the composition of the collectives is shown in Tab. 46.

4.2.3 Pooled Study

Further, a pooled sample set was generated from Munich (MUC) and Swedish (SWE) cohorts described above. Similar allele distribution was given in both cohorts, which was checked before pooling. The association pooled study consisted of a total of 1611 Caucasian subjects comprising 690 individuals with a diagnosis of probable AD (mean age, 70.7 years ± standard deviation 8.4; age at onset, 68.6 years ± 8.1; 41% males) and 921 cognitively healthy controls (mean age, 71.2 years ± 10.4; 41% males).

A detailed table on the composition of the collectives is shown in Tab. 46.

4.2.4 Sib-Pair Study

Finally, the sib-pairs cohort consisted of 735 Caucasian subjects, from 287 families, consisting of 297 AD cases (mean age, 72.2 years ± 9.1; age at onset, 69.0 years ± 9.1; 41% males) and 438 cognitively healthy controls (mean age, 66.8 years ± 12.0; 44% males). The subjects were recruited and phenotyped in collaboration with 21 hospitals (see Tab. 15 below) from Bavaria. In addition, subjects were appealed for contribution in the study by the press. Selection of the sib-pairs was carried out on the basis of one affected sibling for which AD was diagnosed according to the NINCDS-ADRDA criteria.

A detailed table on the composition of the collectives is shown in Tab. 46.

Tab. 15: Participating clinics in the sib-pairs cohort study

1.	Klinik für Psychiatrie und Psychotherapie der TUM, Klinikum rechts der Isar
2.	Psychiatrische Klinik, Agatharied
3.	Bezirkskrankenhaus Ansbach, Klinik für Psychiatrie und Psychotherapie
4.	Psychiatrische Klinik, Bezirkskrankenhaus Augsburg
5.	Neurologische Klinik, Bad Aibling
6.	Bezirkskrankenhaus Bayreuth, Klinik für Psychiatrie und Psychotherapie
7.	Klinik für Psychiatrie, Bezirkskrankenhaus Gabersee
8.	Bezirkskrankenhaus Günzburg, Fachkrankenhaus für Psychiatrie
9.	Psychiatrische Klinik am Klinikum Ingolstadt
10.	Bezirkskrankenhaus Kaufbeuren, Fachkrankenhaus für Psychiatrie
11.	Bezirkskrankenhaus Landshut, Klinik für Psychiatrie und Psychotherapie
12.	Bezirkskrankenhaus Lohr am Main, Krankenhaus für Psychiatrie und Psychotherapie
13.	Psychiatrische Klinik, Bezirksklinikum Mainkofen
14.	Neurologische Klinik, Städt. Krankenhaus Bogenhausen in München
15.	Abteilung für Akutgeriatrie, Krankenhaus Neuperlach in München
16.	Klinik und Poliklinik für Psychiatrie der Universität Regensburg
17.	Bezirkskrankenhaus Taufkirchen
18.	Universitätsklinikum Ulm, Poliklinik für Neurologie
19.	Bezirkskrankenhaus Schloss Werneck
20.	Bezirkskrankenhaus Haar in München
21.	Universitätsklinik Freiburg

4.2.5 Functional Study

Functional analyses of selected SNPs, including gene expression, Western blot and differential Aβ isoform analysis, were carried out in 20 brain (frontal cortex) samples (Department Neuropathology, LMU München) unrelated to all aforementioned cohorts. These samples comprised 12 individuals with a diagnosis of probable AD (Braak stages III – V) and 8 cognitively healthy controls (Braak stages 0 – II). The clinical diagnosis of probable AD was established as described above.

Further functional analyses, Aβ42 levels, were undertaken in a total of 91 cerebro-spinal fluid (CSF) samples, sourced from the Munich case-control cohort. A detailed table on the composition of the collectives is shown in Tab. 47.

5 Results

In the present work 216 SNPs genes (see below) were analyzed and evaluated. Of these, 132 SNPs were tested for their association with AD and covered the following genes:

- the *APP* gene itself
- *BACE1* and *ADAM10*; (genes involved in the processing of APP and therefore in the amount of present Aβ)
- and the *ABCA1* and *LIPC* genes (involved in the lipid metabolism).

5.1 Analysis of SNPs from *APP*

The *APP* gene is located in the region 21q21.3 and contains at least 18 exons within a 240 kilobases region. APP plays a major role during AD pathogenesis, as described before in detail (see Introduction, § 1.4.1). Although *APP* is a strong functional and positional candidate, only one of the aforementioned studies (Nowotny, Simcock et al. 2007) used a fine mapping approach of SNPs across the entire gene. Thus, in a large German case-control cohort, the potential association of the *APP* gene in AD was thoroughly investigated by utilizing a fine mapping approach across the entire *APP* gene. When required, results underwent a replication in a second large independent case-control group as well as an independent sib-pairs cohort. Additionally, associated SNPs underwent further analyses to elucidate their potential functional implications, including their association with altered APP gene expression and effects on Aβ levels.

5.1.1 Genotyping of SNPs from *APP*

After researching several databanks, 73 SNPs were selected (Tab. 16). From these, 39 could be confirmed to be polymorph in the Munich collective, i.e. they achieved a sufficient genotype frequency (>80%) and showed no deviation from the HWE in the controls (Tab. 17 and Fig. 13). Incorporating results with insufficient genotype frequencies can lead to falsified distributions of the formerly matched cases and controls cohorts. Deviations from the HWE can be a sign of erroneous genotyping or a deficient population structure.

Tab. 16: Selected SNPs from the chromosomal region covering the *APP* gene.

SNPs that yielded insufficient genotype frequencies (<80 %), significant deviations from HWE and minor allele frequencies <0.001 were not taken into account for the following statistical evaluations. SNPs passing all aforementioned criteria are highlighted in bold letters. Alleles are reported in alphabetical order as found on http://snpper.chip.org.

SNP ID NCBI	SNP Position NCBI	Alleles	Role
rs2040124	**26150106**	**C/G**	**Intergenic**
rs2829955	**26155219**	**C/G**	**Intergenic**
rs6516704	**26161592**	**A/T**	**Intergenic**
rs6516708	**26166780**	**G/T**	**3' UTR**
rs2242682	26170300	C/T	3' UTR
rs214483	26174597	A/G	3' UTR
rs11549660	**26175202**	**A/G**	**Exon**
rs373521	**26179531**	**A/C**	**Intron**
rs2070653	**26185464**	**C/T**	**Intron**
rs1800557	26185978	C/T	Coding exon
rs2829975	**26190408**	**G/T**	**Intron**
rs1700999	26195753	G/T	Intron
rs1783016	**26201909**	**C/T**	**Intron**
rs2829983	**26206976**	**G/T**	**Intron**
rs6516711	26212927	G/T	Intron
rs7510374	26219072	C/T	Intron
rs214481	26224519	C/G	Intron
rs9981102	26229733	A/T	Intron
rs2829993	26235536	A/C	Intron
rs216772	26241140	A/T	Intron
rs216781	**26246376**	**C/T**	**Intron**
rs1967945	26248681	A/T	Intron (boundary)
rs2186301	**26252378**	**A/G**	**Intron**
rs216762	**26258039**	**C/T**	**Intron**
rs7277169	26264038	C/G	Intron
rs3737413	26270243	A/G	Intron (boundary)
rs4817076	26275931	C/T	Intron
rs762479	**26281147**	**C/G**	**Intron**
rs7278134	26287627	A/T	Intron
rs9978555	**26292577**	**A/G**	**Intron**
rs10084562	26298651	C/G	Intron
rs2409166	26304288	C/G	Intron
rs2830005	26309855	A/C	Intron
rs9941877	**26314397**	**C/T**	**Intron**
rs2830012	**26319478**	**A/G**	**Intron**
rs2830019	**26324683**	**C/T**	**Intron**
rs768039	**26329891**	**C/T**	**Intron**
rs7275712	26334570	C/T	Intron
rs3787644	**26340941**	**A/G**	**Intron**
rs2070655	**26345432**	**A/C**	**Intron (boundary)**
rs3991	**26350127**	**G/T**	**Intron**
rs2830035	**26355571**	**A/G**	**Intron**
rs743532	**26360372**	**C/G**	**Intron**
rs1041420	**26365521**	**A/G**	**Intron**
rs2830042	26370641	C/T	Intron
rs17001673	26376058	A/G	Intron
rs2830046	**26380577**	**A/G**	**Intron**
rs8130594	**26384648**	**C/G**	**Intron**

rs6516725	26388197	C/G	Intron
rs2830053	**26393582**	**C/T**	**Intron**
rs4817089	26398796	A/T	Intron
rs2234989	26404833	C/T	Intron
rs11549661	26406210	C/T	Coding exon
rs2246115	**26409179**	**C/G**	**Intron**
rs2830065	26415397	G/T	Intron
rs2830069	**26420461**	**G/T**	**Intron**
rs462278	**26426266**	**A/G**	**Intron**
rs2830082	**26431745**	**A/G**	**Intron**
rs1788283	**26436966**	**A/C**	**Intron**
rs2830092	**26442148**	**A/C**	**Intron**
rs6516727	**26447377**	**A/G**	**Intron**
rs2830099	**26452481**	**C/G**	**Intron**
rs4817090	26456132	C/T	Intron
rs465984	**26460990**	**C/T**	**Intron**
rs459543	26464920	C/G	Exon
rs466433	26465834	A/G	Promoter
rs364048	26465912	C/T	Promoter
rs2156079	26470201	A/G	Promoter
rs1235885	**26473927**	**C/T**	**Promoter**
rs370293	26474586	C/T	Promoter
rs8131261	**26479553**	**C/T**	**Intergenic**
rs2830105	26484522	C/T	Intergenic
rs460029	26488824	C/T	Intergenic

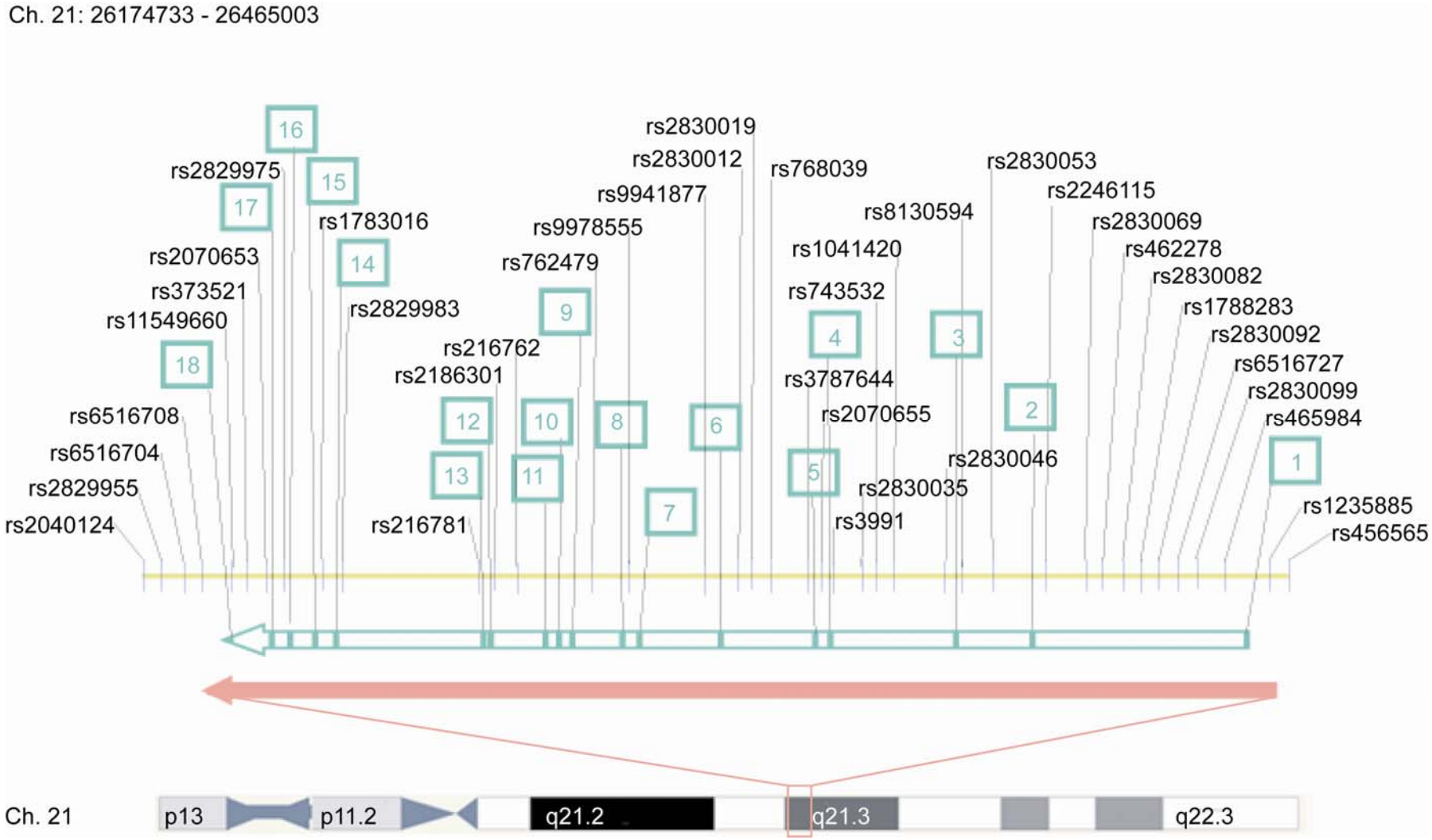

Fig. 13: Schematic of the *APP* gene.

The positions of the analyzed SNPs (shown with rs numbers) and exons in the *APP* gene are shown. Exons are marked with green bars on the forward strand from left to right. The arrow shows direction of transcription.

The subsequent allelic tests yielded an association between 4 SNPs and AD in the complete Munich collective. The strongest association signal was found for the intronic SNP rs3787644 (p = 0.015, # 20). A detailed analysis stratified for age at onset yielded a weaker p-value of 0.023 in subjects with EOAD and no significance was detected in subjects with LOAD (p = 0.323).

The association with AD was also confirmed for the non *ApoE* ε4 sub-collective, but with a p-value of 0.034. The risk allele for SNP # 20 (rs3787644) was the major allele C, with a higher allele frequency in the cases. Significant p-values were also detected for the intronic SNPs rs9941877 (p = 0.018, # 17), rs6516727 (p = 0.035, # 35) and rs2830099 (p = 0.049, # 36). Unlike SNP # 16 and 36, the result for SNP # 35 could be confirmed in subjects with LOAD, although with a weaker association as for the whole collective (p = 0.041). For SNPs # 35 and 36 the major alleles (T and G) were determined as risk alleles with a higher frequency in the AD cases. For SNP # 16 the minor allele (A) was associated with appearance of AD, while the major allele was more prominent in the controls.

Several additional SNPs were calculated to have moderate significance in the sub-collectives divided by age at onset, which had shown no significance in the whole Munich cohort: One SNP (# 19) in subjects with EOAD and 7 more SNPs (# 14, 15, 21, 22, 24, 25 and 34) in subjects with LOAD.

For the historical role of the ApoE gene, the samples were also stratified according to the presence or absence of the *ApoE* ε4 allele. However, no major differences were observed between both groups and yielded no further significant p-values.

Results from allelic tests of all SNPs performed for the Munich collective are depicted in Tab. 17.

Tab. 17: Results from allelic tests for the *APP* gene.

Significance of allelic association was calculated using a $\chi 2$-test, as implemented in Haploview version 4.2. The statistical analyses were carried out for the whole collective as well as for sub-collectives divided by age/onset (EOAD ≤ 65 years, LOAD > 65 years) and presence of at least one ApoE4 allele (ApoE4 = at least one ApoE4 allele, nonApoE4 = no ApoE4 allele) for cases and controls. All of the listed SNPs yielded genotype frequencies of at least 80 %, no significant deviation from HWE and minor allele frequencies were at least 0.001. Significant results are highlighted in bold letters.

#	SNP	Allelic test results						
		Associated allele	Major allele frequency MUC complete (cases, controls)	Allelic p-values				
				MUC complete (N=728)	EOAD subcollective (N=281)	LOAD subcollective (N=444)	*ApoE* ε4 subcollective (N = 295)	non *ApoE* ε4 subcollective (N = 433)
1	rs2040124	C	0.989, 0.986	0.558	0.975	0.511	0.883	0.921
2	rs2829955	G	0.525, 0.538	0.638	0.879	0.641	0.381	0.808
3	rs6516704	A	0.542, 0.540	0.946	0.855	0.865	0.714	0.605
4	rs6516708	A	0.507, 0.527	0.473	0.854	0.533	0.357	0.993
5	rs11549660	T	0.998, 1.000	0.247	0.161	0.929	0.454	-
6	rs373521	G	0.571, 0.566	0.845	0.932	0.544	0.638	0.992
7	rs2070653	C	0.743, 0.760	0.476	0.618	0.858	0.196	0.929
8	rs2829975	C	0.876, 0.889	0.470	0.362	0.583	0.251	0.160
9	rs1783016	G	0.757, 0.734	0.334	0.102	0.280	0.663	0.371
10	rs2829983	C	0.881, 0.896	0.391	0.867	0.389	0.849	0.801
11	rs216781	G	0.927, 0.940	0.325	0.757	0.216	0.427	0.601
12	rs2186301	C	0.977, 0.970	0.382	0.969	0.859	0.747	0.439
13	rs216762	G	0.533, 0.525	0.760	0.489	0.105	0.683	0.716
14	**rs762479**	G	0.772, 0.808	0.108	0.652	**0.016**	0.654	0.237
15	**rs9978555**	C	0.953, 0.963	0.348	0.324	**0.039**	0.341	0.411
16	**rs9941877**	A	0.688, 0.746	**0.018**	0.256	0.287	0.057	0.266

17	rs2830012	T	0.669, 0.710	0.099	0.160	0.160	0.125	0.542
18	rs2830019	G	0.959, 0.967	0.462	0.463	0.517	0.402	0.650
19	**rs768039**	C	0.701, 0.654	0.062	**0.017**	0.119	0.860	0.053
20	**rs3787644**	C	0.674, 0.611	**0.015**	**0.023**	0.323	0.206	**0.034**
21	**rs2070655**	C	0.713, 0.670	0.089	0.065	**0.036**	0.813	0.087
22	**rs3991**	G	0.804, 0.764	0.072	0.766	**0.044**	0.059	0.657
23	rs2830035	C	0.762, 0.731	0.187	0.872	0.531	0.357	0.522
24	**rs743532**	G	0.957, 0.945	0.308	0.405	**0.037**	0.136	0.862
25	**rs1041420**	T	0.728, 0.765	0.121	0.703	**0.033**	0.131	0.673
26	rs2830046	T	0.766, 0.729	0.109	0.961	0.481	0.100	0.723
27	rs8130594	C	0.689, 0.690	0.942	0.650	0.850	0.743	0.680
28	rs2830053	A	0.896, 0.910	0.364	0.161	0.259	0.991	0.207
29	rs2246115	G	0.848, 0.879	0.104	0.243	0.629	0.764	0.116
30	rs2830069	C	0.897, 0.912	0.353	0.293	0.171	0.899	0.154
31	rs462278	C	0.971, 0.977	0.503	0.431	0.507	0.670	0.819
32	rs2830082	T	0.896, 0.912	0.325	0.365	0.155	0.937	0.202
33	rs1788283	G	0.567, 0.541	0.350	0.959	0.482	0.238	0.841
34	**rs2830092**	T	0.896, 0.914	0.257	0.262	**0.011**	0.948	0.108
35	**rs6516727**	T	0.711, 0.659	**0.035**	0.644	**0.041**	0.104	0.200
36	**rs2830099**	G	0.741, 0.693	**0.049**	0.433	0.171	0.142	0.284
37	rs465984	A	0.972, 0.977	0.536	0.385	0.173	0.663	0.830
38	rs1235885	G	0.961, 0.965	0.719	0.365	0.395	0.161	0.664
39	rs456565	T	0.569, 0.551	0.488	0.864	0.864	0.216	0.688

For all of the 39 SNPs (Tab. 17), genotypic analyses were performed using logistic regression with gender and age at onset as covariates. In the complete Munich collective the same 4 SNPs - found before by using allelic tests - could be shown to be associated with AD. The strongest association signal was again found for the intronic SNP rs3787644 (# 20), with an even more significant p-value of 0.006. A before, detailed analyses stratified by age at onset yielded an equally strong association in subjects with EOAD ($p = 0.007$) and again no significance in subjects with LOAD. The association with AD was also confirmed for the non *ApoE* ε4 sub-collective with a p-value of 0.017. The p-values detected for the intronic SNPs rs9941877 (# 17), rs6516727 (# 35) and rs2830099 (# 36) were 0.031, 0.049 and 0.030, respectively. In the genotypic approach all three SNPs could be confirmed in subjects with LOAD with stronger association signals as for the whole collective (# 17: $p = 0.016$, # 35: $p = 0.014$ and # 35: $p = 0.011$. Furthermore, this stratification identified additional association signals for SNPs 9, 17, 19 (EOAD group) and 15 (LOAD group).

All formerly determined risk alleles were confirmed using genotypic tests. Results from genotypic tests of all SNPs performed for the Munich collective are depicted in Tab. 18. Stratification for presence of the *ApoE* ε4 allele was also performed for the genotypic approach. One additional significant signal was found for SNP # 2 (rs2829955), but only with a very weak borderline value of 0.05. Results for stratification for presence of *ApoE* ε4 allele are presented in the Appendix (§ 7.3.1, Tab. 48).

Tab. 18: Results from genotypic tests for the *APP* gene.

Genotypic associations were tested for significance using logistic regression, all as implemented in STATA/SE 9.2. The statistical analysis was carried out for the whole collective as well as for sub-collectives divided by age/onset (EOAD ≤ 65 years, LOAD > 65 years) and presence of at least one ApoE4allele (ApoE4 = at least one ApoE4 allele, nonApoE4 = no ApoE4 allele) for cases and controls. Gender and age/onset were taken into account as covariates. Significant results are highlighted in bold font. Note that SNP # 5 could not be evaluated, due to absence of the minor allele in the control group.

#	SNP	Alleles (major/ minor)	Genotypic test results					
			Genotype frequencies MUC complete (cases, controls)			AD affection genotypic p-values for major allele unless otherwise noted (associated allele); OR (95%CI) with covariates (sex and onset)		
			AA	Aa	aa	MUC complete (N=728)	EOAD subcollective (N=281)	LOAD subcollective (N=444)
1	rs2040124	C/G	0.981, 0.975	0.016, 0.021	0.002, 0.004	0.595	0.634	0.913
2	rs2829955	C/G	0.290, 0.290	0.470, 0.497	0.240, 0.214	0.931	0.780	0.821
3	rs6516704	A/T	0.305, 0.288	0.474, 0.505	0.221, 0.207	0.596	0.779	0.742
4	rs6516708	C/A	0.267, 0.281	0.481, 0.491	0.252, 0.228	0.687	0.896	0.583
6	rs373521	G/T	0.340, 0.317	0.464, 0.498	0.197, 0.185	0.577	0.582	0.587
7	rs2070653	T/C	0.543, 0.572	0.400, 0.375	0.057, 0.052	0.394	0.826	0.381
8	rs2829975	A/C	0.770, 0.787	0.211, 0.202	0.019, 0.010	0.672	0.302	0.948
9	**rs1783016**	G/A	0.575, 0.552	0.361, 0.365	0.064, 0.083	0.660	**0.045 (G); 1.64 (1.01-2.7)**	0.333
10	rs2829983	A/C	0.779, 0.802	0.205, 0.186	0.016, 0.010	0.489	0.819	0.334
11	rs216781	A/G	0.861, 0.880	0.132, 0.120	0.007, 0.000	0.482	0.734	0.344
12	rs2186301	C/T	0.957, 0.940	0.040, 0.060	0.002, 0.000	0.210	0.770	0.342
13	rs216762	G/A	0.274, 0.286	0.519, 0.477	0.208, 0.237	0.788	0.962	0.518
14	rs762479	C/G	0.598, 0.647	0.349, 0.322	0.053, 0.031	0.207	0.703	0.137
15	**rs9978555**	T/C	0.909, 0.930	0.089, 0.066	0.002, 0.003	0.271	0.374	**0.018 (C); 3.33 (1.3-10.0)**
16	**rs9941877**	G/A	0.469, 0.550	0.439, 0.391	0.093, 0.059	**0.031 (A); 1.39 (1.03-2.0)**	0.680	**0.016 (A); 1.64 (1.1-2.5)**
17	**rs2830012**	C/T	0.455, 0.502	0.427, 0.417	0.118, 0.081	0.236	**0.032 (T); 2.58 (1.1-6.1)**	0.094
18	rs2830019	A/G	0.920, 0.937	0.077, 0.060	0.002, 0.004	0.425	0.451	0.052

19	**rs768039**	C/T	0.495, 0.439	0.411, 0.429	0.094, 0.131	0.155	**0.032 (C); 1.69 (1.05-2.7)**	0.947
20	**rs3787644**	C/T	0.472, 0.366	0.406, 0.489	0.123, 0.144	**0.006 (C); 1.55 (1.1-2.1)**	**0.007 (C); 1.99 (1.2-3.3)**	0.109
21	rs2070655	C/A	0.524, 0.471	0.374, 0.398	0.102, 0.131	0.174	0.094	0.660
22	rs3991	G/T	0.633, 0.573	0.341, 0.381	0.026, 0.045	0.114	0.969	0.063
23	rs2830035	C/T	0.560, 0.520	0.404, 0.423	0.035, 0.057	0.243	0.772	0.074
24	rs743532	G/C	0.913, 0.893	0.087, 0.103	0.000, 0.003	0.315	0.349	0.458
25	rs1041420	C/T	0.525, 0.579	0.407, 0.372	0.068, 0.049	0.127	0.983	0.059
26	rs2830046	T/C	0.590, 0.532	0.354, 0.394	0.056, 0.074	0.114	0.967	0.077
27	rs8130594	G/C	0.477, 0.473	0.422, 0.434	0.100, 0.093	0.882	0.444	0.923
28	rs2830053	G/A	0.803, 0.831	0.185, 0.158	0.012, 0.012	0.327	0.090	0.774
29	rs2246115	C/G	0.725, 0.778	0.241, 0.201	0.034, 0.021	0.119	0.090	0.244
30	rs2830069	A/C	0.807, 0.835	0.181, 0.154	0.012, 0.011	0.350	0.235	0.537
31	rs462278	T/C	0.944, 0.953	0.054, 0.047	0.002, 0.000	0.597	0.533	0.190
32	rs2830082	C/T	0.806, 0.834	0.180, 0.156	0.014, 0.010	0.348	0.285	0.406
33	rs1788283	G/T	0.286, 0.270	0.562, 0.541	0.153, 0.189	0.541	0.808	0.468
34	rs2830092	G/T	0.803, 0.838	0.185, 0.151	0.012, 0.011	0.241	0.207	0.338
35	**rs6516727**	T/C	0.486, 0.418	0.451, 0.481	0.063, 0.101	**0.049 (T); 1.36 (1.001-1.8)**	0.376	**0.014 (T); 2.44 (1.3-5.0)**
36	**rs2830099**	G/C	0.530, 0.474	0.421, 0.439	0.049, 0.088	**0.030 (G); 1.89 (1.1-5.0)**	0.227	**0.011 (G); 2.78 (1.3-5.0)**
37	rs465984	G/A	0.946, 0.954	0.052, 0.046	0.002, 0.000	0.643	0.490	0.171
38	rs1235885	A/G	0.925, 0.933	0.073, 0.063	0.002, 0.004	0.675	0.520	0.143
39	rs8131261	T/C	0.302, 0.296	0.534, 0.509	0.164, 0.195	0.805	0.916	0.864

The 39 SNPs covering the complete genetic region of the *APP* gene (330 kb, average inter-marker distance of 8.7 kb) determined the linkage disequilibrium (LD) structure, estimated by D' and r^2 and explored haplotype associations as implemented in Haploview. Within the genotyped region 5 LD blocks were identified on basis of D' and r^2 values (Fig. 14). For each of the LD blocks, the haplotypes were calculated as depicted in the table below (Tab. 19). The 4 significant markers identified in the Munich collective before were found in two different LD blocks. SNP # 16 (rs9941877) is located at the border of block 2. Since allocation of SNP # 16 to this block is not distinct (D' values below 0.9, but well above 0.8) correlations with the adjacent SNPs were tested. No correlation was found with the direct neighbor SNP # 15 (rs9978555, $r^2 = 0.04$, D' = 0.66), but a slight correlation was shown with direct neighbor SNP # 17 (rs2830012, $r^2 = 0.60$, D' = 0.82). SNP # 20 is located in the same block (block 2), SNPs # 34 and 35 in block 5.

In total, the calculations predicted haplotypes with frequencies above 5 % within block 2, spanning SNPs # 16-21. One of the haplotypes showed an association with AD (p = 0.037) with a frequency of 31.6 % in the controls and 26.5 % in the cases. 3 haplotypes with frequencies above 5 % were estimated within block 5, but none of the estimated haplotypes showed an association with AD.

The signal in LD block 2 was the only association found during analysis of haplotype blocks.

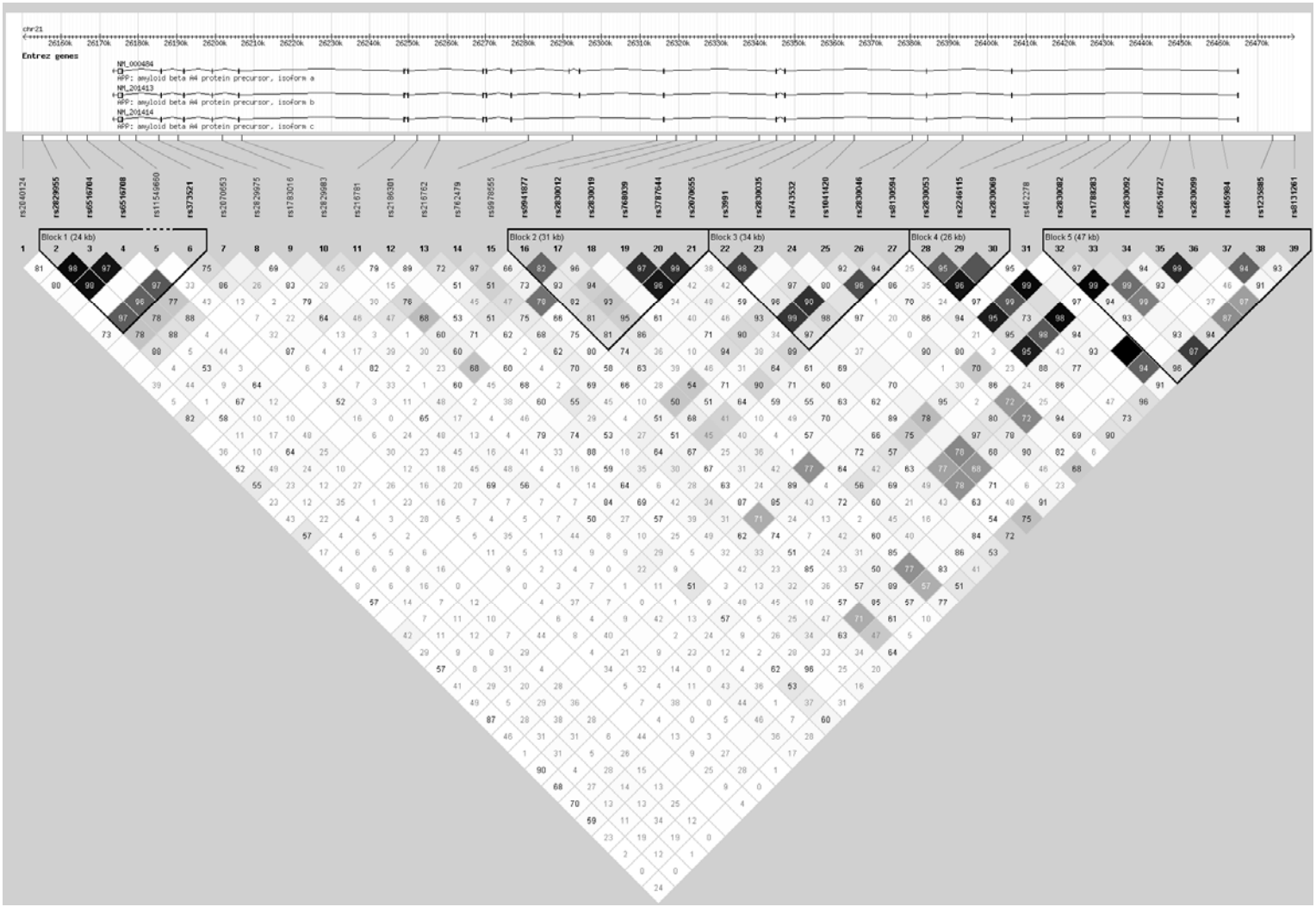

Fig. 14: LD structure of the *APP* gene, including the haplotype block structure.

The depicted values are used to represent D' whereas colors were used to represent r^2 (D' values of 1.0 are never shown - the box is empty; black = high r^2 value, white = low r^2 value). The blocks followed the haplotype block definition by Gabriel et al., (Gabriel, Schaffner et al. 2002) as implemented within Haploview 4.2.

Tab. 19: Haplotype blocks and frequencies for *APP* in Munich collective

Haplotype ID	Sequence	Frequency (Case/Control)	P-values
Block 1			
1.1	GTAG	0.465, 0.455	0.705
1.2	CACT	0.419, 0.426	0.789
1.3	CACG	0.091, 0.102	0.510
Block 2			
2.1	GCACCC	0.323, 0.308	0.534
2.2	GCATTA	0.265, 0.316	**0.037**
2.3	ATACCC	0.223, 0.192	0.158
2.4	GTACCC	0.058, 0.059	0.902
Block 3			
3.1	GCGCTG	0.426, 0.399	0.310
3.2	GCGTTC	0.260, 0.236	0.305
3.3	TTGCCG	0.192, 0.223	0.157
Block 4			
4.1	GCA	0.844, 0.876	0.086
4.2	AGC	0.098, 0.088	0.504
Block 5			
5.1	CGGTGGAT	0.543, 0.517	0.337
5.2	CTGCCGAC	0.237, 0.268	0.177
5.3	TTTTGGAC	0.100, 0.079	0.192

Significant p values are highlighted in **bold** letters.

The final analysis performed for the Munich cohort was a 2- and 3-marker sliding window approach to identify SNPs that contributed association signals (Fig. 15). In general two marker peaks were observed spanning SNPs # 14-24 and # 33-37. The strongest association was observed in the 3-marker haplotype 20.1 generated by SNPs # 20-22 ($p = 0.0028$). Additional strong association signals were observed in 3-marker haplotypes 14.1 from SNPs # 14-16 ($p = 0.0057$) and 21.1 from # 21-23 ($p = 0.0119$) and in the 2-marker haplotype 15.1 generated by SNPs # 15 and 16 ($p = 0.0096$). Within the two regions of the marker peaks haplotype associations were clearly higher than those of the single markers.

A 2- and 3-marker sliding window approach was also performed for the stratified sub-collectives divided by age at onset or presence of *ApoE* ε4 allele. Only the first marker peak from the whole Munich cohort could be observed in subjects with EOAD, but was shorter than in the whole cohort, only spanning from SNPs # 17 – 21. In the LOAD sub-collective the first peak was subdivided into two peaks, spanning from SNPs # 14 – 16 and # 20 – 28. The second peak was even broader compared to the one observed in the complete Munich cohort, spanning from SNPs # 31 – 37. No real differences between the two sub-cohorts that were divided by presence or absence of the *ApoE* ε4 allele were found. In both sub-cohorts, only

the first peak was found, but merely spanning from SNPs # 20 – 22 (*ApoE* ε4) or # 18 – 21 (non-*ApoE* ε4), respectively.

Detailed results from the 2- and 3-marker sliding window approach are depicted in the Appendix, as well as graphs for the sub-collectives (§ 7.4.1). Haplotypes with frequencies below 5% were not taken into account, due to their unlikely occurrence.

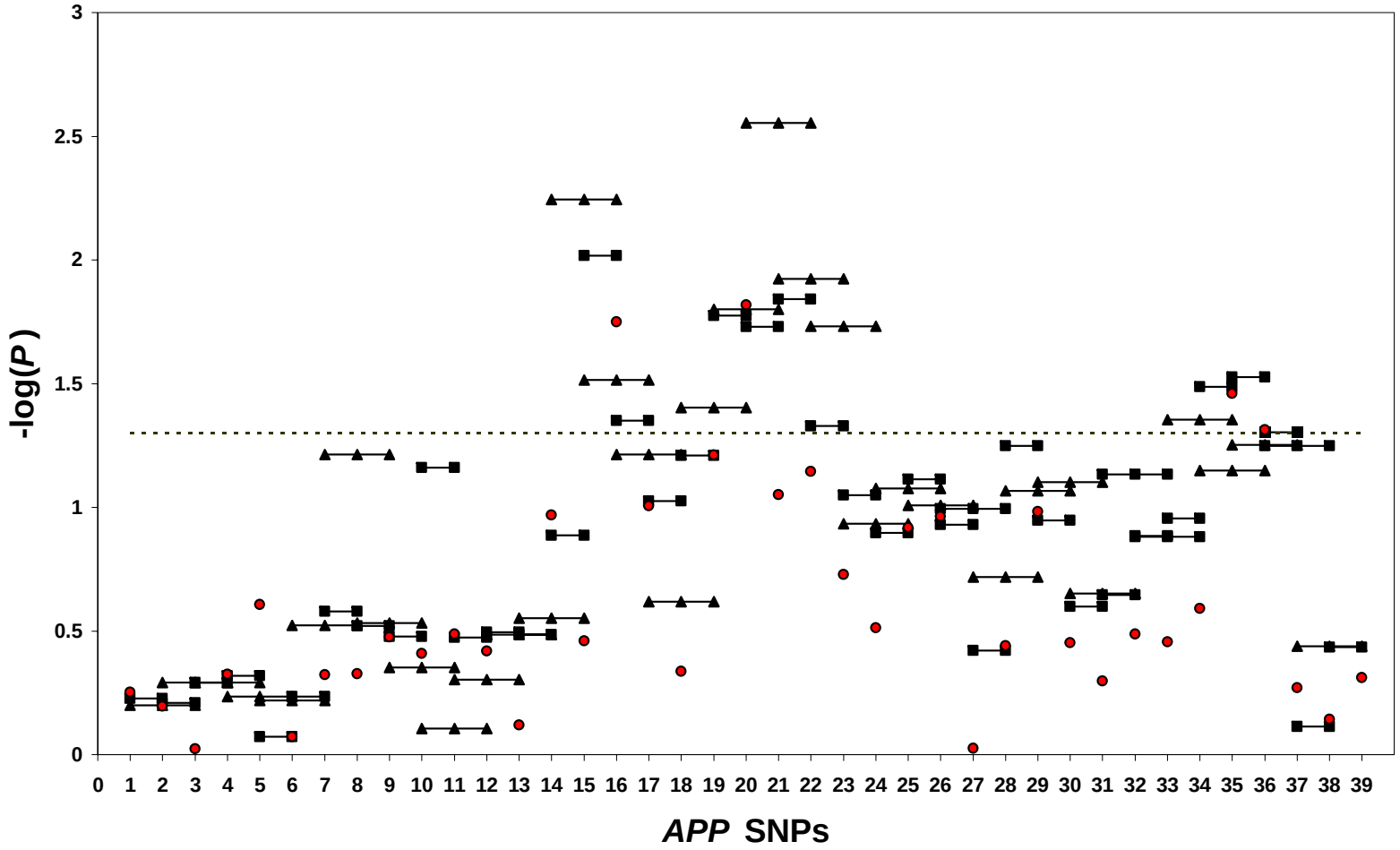

Fig. 15: Sliding window analysis of 2- and 3-marker haplotypes for *APP*

Red circles represent -log p values for single marker SNPs genotyped. Lines between solid squares represent the -log p value of 2-marker haplotypes. Lines between solid triangles mark -log p values for 3-marker haplotypes. All 2- and 3-marker haplotypes were calculated with Haploview. The dotted lines at a -log p value of approximately 1.3 demarcate the threshold of p = 0.05. Numbers on the X-axis correspond to the numbering assigned to SNPs by Haploview.

5.1.2 Replication of Association Results in Independent Studies

Based on the analysis of the Munich cohort (MUC) a selection of SNPs (# 15-23, 25, 35 and 36) was chosen for replication in independent sub-populations. In the scope of this study a large Swedish case-control cohort (SWE, N = 883) and a German discordant sib-pairs cohort (N = 735) were available. A subsequent pooling analysis of the Munich and Swedish cohort (pooled = MUC + SWE, N = 1611) was performed to increase power. Results from SNP # 19 had to be excluded, due to possible miscalling during genotyping in the Swedish sample, indicated by severe changes in the genotype frequencies.

In the Swedish sample, association signals were replicated in SNPs # 17, 25 and 36. Overall, SNPs # 17 and 20 had relatively weak association signals ($p < 0.05$) in the pooled analysis, whereas stronger signals ($p < 0.005$) were observed for SNPs # 25 and 36. Stratification of the pooled sample strengthened the association of SNP # 20, but only in the EOAD group. SNPs # 25 and 36 had equivalent or slightly weaker association signals, respectively, but limited to the LOAD group. Detailed results of the genotypic tests are depicted in Tab. 20. Results of allelic test are shown in the Appendix (§ 7.3.1, Tab. 49).

As with the Munich cohort, analysis of 2- and 3-marker loci in the Swedish and pooled cohorts were performed for SNPs # 15 – 25 and # 35-36, which are located next to each other. Analysis of 2- and 3-marker loci, previously identified in the Munich cohort, replicated the haplotype associations for SNPs 20-22 (Sweden, $p = 0.0027$) and SNPs # 35-36 (Sweden, $p = 1.35 \times 10^{-6}$), however, the haplotype generated by SNPs # 21-23 failed to reach significance (see Appendix, § 7.4.1, Tab. 69 - Tab. 72, Fig. 37 and Fig. 38).

Due to the limited amount of available DNA, analyses of SNPs in the sib-pairs cohort were only performed for the 4 SNPs that had shown significance in the complete Munich cohort (SNPs # 16, 20, 35 and 36) as well as for the adjacent SNPs of the strongest association marker - SNP # 20 - (SNPs # 19 and 21). The experiments failed to replicate signals for SNPs # 20 and 36; however, additional signals were identified at SNP # 16 (rs9941877; FBAT, $p < 0.05$) and SNP 35 (rs6516727; FBAT, $p < 0.05$). Results of the tests are depicted in Tab. 20.

Tab. 20: Results of replication for selected SNPs of *APP* in independent studies – part 1

#	SWE (N=883)			pooled Population (MUC+SWE) (N=1611)		
	AD affection genotypic p-values for major allele unless otherwise noted (associated allele); OR (95%CI) with covariates (sex and onset)			AD affection genotypic p-values for major allele unless otherwise noted (associated allele); OR (95%CI) with covariates (sex, onset, population)		
	SWE complete (N=883)	EOAD subcollective (N=220)	LOAD subcollective (N=661)	pooled complete (N=1611)	EOAD subcollective (N=501)	LOAD subcollective (N=1105)
15	0.224	0.492	0.559	0.934	0.578	0.327
16	0.223	0.779	0.535	0.142	0.441	0.177
17	**0.036 (T); 1.46 (1.3-2.1)**	0.403	0.109	**0.016 (T); 1.55 (1.1-2.2)**	**0.038 (T); 1.865 (1.03-3.4)**	0.934
18	0.454	0.370	0.696	0.775	0.526	0.322
20	**0.988**	0.648	**0.030 (T); 1.85 (1.1-3.2)**	**0.045 (C); 1.25 (1.005-1.6)**	**0.006 (C); 1.69 (1.2-2.5)**	0.523
21	**0.028 (A); 1.76 (1.1-2.9)**	0.062	**0.003 (A); 2.44 (1.4-4.4)**	0.076	**0.006 (C); 1.69 (1.2-2.5)**	0.700
22	0.606	0.880	0.727	0.088	0.926	0.092
23	**0.003 (T); 2.33 (1.3-4.0)**	0.104	**0.005 (T); 2.49 (1.3-4.8)**	0.056	0.437	0.157
25	**0.002 (T); 2.37 (1.4-4.1)**	0.799	**0.013 (T); 2.33 (1.2-4.6)**	**0.001 (T); 1.98 (1.3-3.0)**	0.818	**0.005 (T); 2.22 (1.3-3.9)**
35	0.863	0.768	0.793	0.686	0.628	0.912
36	**0.029 (G); 1.44 (1.04-2.0)**	0.224	0.091	**0.004 (G); 1.38 (1.1-1.7)**	0.065	**0.017 (G); 1.42 (1.1-1.9)**

Tab. 20: Results of replication for selected SNPs of *APP* in independent studies – part 2

#	SNP	SIB pairs (N=735)	
		fbat100	fbat010
16	**rs9941877**	**0.026**	**0.012**
19	rs768039	0.382	0.382
20	rs3787644	0.959	0.628
21	rs2070655	0.638	0.449
35	**rs6516727**	0.114	**0.030**
36	rs2830099	0.377	0.173

5.1.3 Statistical Analyses of Clinical Measures for *APP*

Linear regression analysis of clinical measures was performed, testing association between genotypes of the SNPs and age at onset and the scores on the Mini-mental State Examination (MMSE) within the whole Munich collective and for the complete set of 39 SNPs. Note that MMSE scores were only available for 416 of the subjects. For the analysis, age at onset and gender were included as covariates to rule out results only driven by group-specific effects. Resulting data revealed associations between SNP # 9 and age at onset (GG, 68.9 ± 10.4 years; GA/AA, 67.1 ± 10.2: p = 0.032) as well as MMSE scores (GG, 17.9 ± 6.2; GA/AA, 19.4 ± 6.5: p = 0.018). SNP # 9 had already yielded a significant p-value when tested for association with AD in the whole Munich cohort after stratification by age at onset within the EOAD sub-collective. The G allele had been associated with a higher risk (compare § 5.1.1, Tab. 18). Association was revealed between several other SNPs (# 13, 28, 30, 32 and 34) and scores on the Mini-mental State Examination (MMSE). However, none of the SNPs that had shown associations before yielded significant results when tested for association with MMSE score. No other SNPs presented with significant associations with clinical measures. Detailed results are reported in the Appendix (§ 7.3.1, Tab. 50 and Tab. 51).

5.1.4 Functional Analyses for *APP* in Brain Samples

In order to determine possible functional effects of SNPs on APP expression and Aβ levels, a preliminary study with 20 brain samples (12 cases, 8 controls) was performed.

As for the replication studies, based on the analysis of the Munich cohort a selection of SNPs (# 15-23, 25, 35 and 36) was chosen for analysis of possible functional effects. These SNPs had delivered association results before and span the two formerly identified marker peak regions (§ 5.1.1, Fig. 15).

The formerly found associations of genotypes with AD were tested in the small study cohort of 20 brain samples. Only one of the SNPs (# 15, rs9978555) confirmed the formerly found association in plasma samples in the used brain samples when tested recessive for the major allele (t-test, p = 0.002). ANOVA analysis yielded no significant results for any of the tested SNPs. Results are presented in the table below (Tab. 21). Due to the small sample size (n = 20) and the explorative character of the functional study, the following investigations

were undertaken anyways, but results have to interpreted with caution.

Tab. 21: Results of statistical analysis of *APP* genotypes in brain samples

#	SNP	p-values from ANOVA (association between genotype and phenotype)	p-values from t-test (recessive for major allele)
15	**rs9978555**	0.264	**0.002**
16	rs9941877	0.787	0.482
17	rs2830012	0.519	0.519
18	rs2830019	-	0.811
19	rs768039	0.811	0.811
20	rs3787644	0.811	0.811
21	rs2070655	0.967	1.000
22	rs3991	0.469	0.482
23	rs2830035	0.220	0.332
25	rs1041420	0.811	0.811
35	rs6516727	0.578	0.388
36	rs2830099	0.772	0.605

- : no ANOVA possible, 2 groups only

5.1.4.1 Gene Expression Analyses for APP in Brain Samples

Using control RNA of defined concentration (Applied Biosystems FirstChoice® Human Brain Reference RNA, 1 mg/mL, Part No. AM6050) a calibration line was determined from which the RNA content of the samples was calculated (Fig. 16).

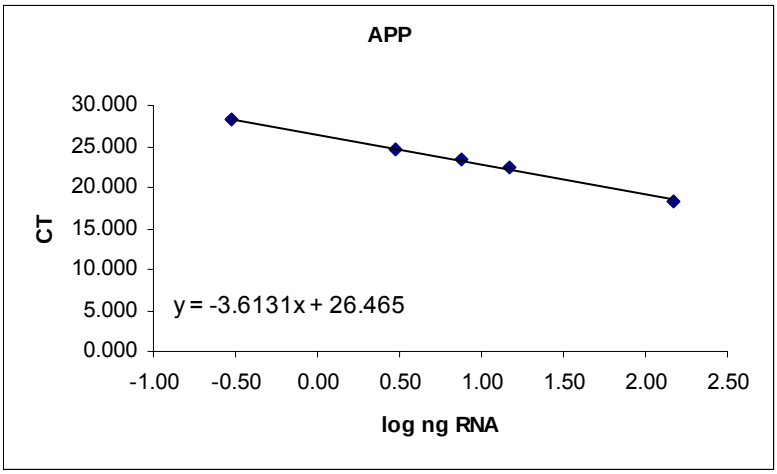

Fig. 16: Calibration line for determination of *APP* mRNA

Gene expression analysis in AD and control brains revealed SNP # 25, which is located in intron 3, to have a significant impact on APP mRNA levels. The C allele was associated with lower mRNA levels, whereas the presence of a T seems to increase APP gene expression (all samples: CC (n=16) 1.69±0.99 Arbitrary Units (AU), CT/TT (n=3) 3.07±1.34 AU; p = 0.049). This association signal strengthened when AD brains alone were examined (AD samples: CC (n=10) 1.68 ± 1.06 AU, CT/TT (n=2) 3.79 ± 0.70; p = 0.025). None of the other SNPs showed significant associations with expression changes. Note that for two SNPs (# 18 and 23) no ANOVA analysis was possible, due to occurrence of two different genotypes only. Therefore, for SNPs # 18 and 23 results of t-test recessive for major allele was performed and results are marked in italic font. The individual results for ANOVA analysis of each SNP are presented in the table below (Tab. 22). A scatter plot depicting the expression changes for SNP # 25 in the AD brains is shown below (Fig. 17).

Tab. 22: Results from the statistical analysis measuring the influence of sequence variation on gene expression levels of *APP*

#	SNP	p-values from ANOVA - association between genotype and APP mRNA level (in AD brains only)
15	rs9978555	0.936
16	rs9941877	0.406
17	rs2830012	0.181
18	rs2830019	*0.083*
19	rs768039	0.414
20	rs3787644	0.414
21	rs2070655	0.392
22	rs3991	0.924
23	rs2830035	*0.267*
25	**rs1041420**	**0.049 (0.025)**
35	rs6516727	0.482
36	rs2830099	0.380

Results in *italic* letters: p-values from t-test, no ANOVA possible, 2 groups only

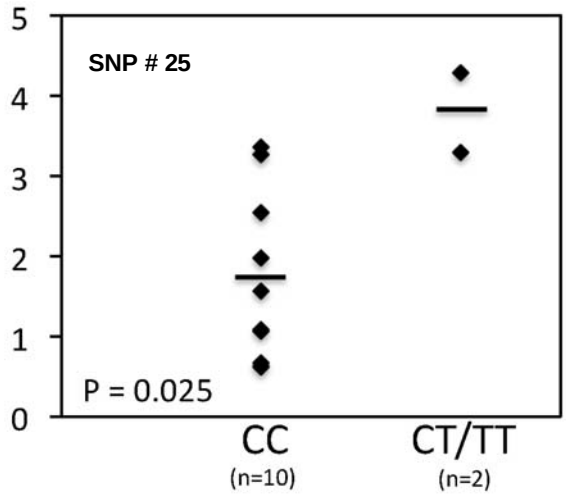

Fig. 17: Effect of rs1041420 (SNP # 25) genotypes on *APP* expression

The y-axis shows the arbitrary units (AU) calculated for each sample, the x-axis represents the determined genotypes. APP expression levels in the brain of AD patients in relation to rs1041420 genotypes were analyzed using ANOVA. Solid lines represent the mean of the measurements per genotype.

5.1.4.2 Western Blot Analysis of Aβ peptides in Brain Samples

A Western Blot was performed for the same 20 brain samples described above. The used primary antibody, WO2, is a monoclonal antibody, which recognises the Aβ peptide and therefore unprocessed APP as well as β-secretase processed APP (CTFβ). It was tested whether protein levels of APP and/or Aβ were elevated in subjects affected with AD in comparison to control brains. The results are shown in the Figure below (Fig. 18).

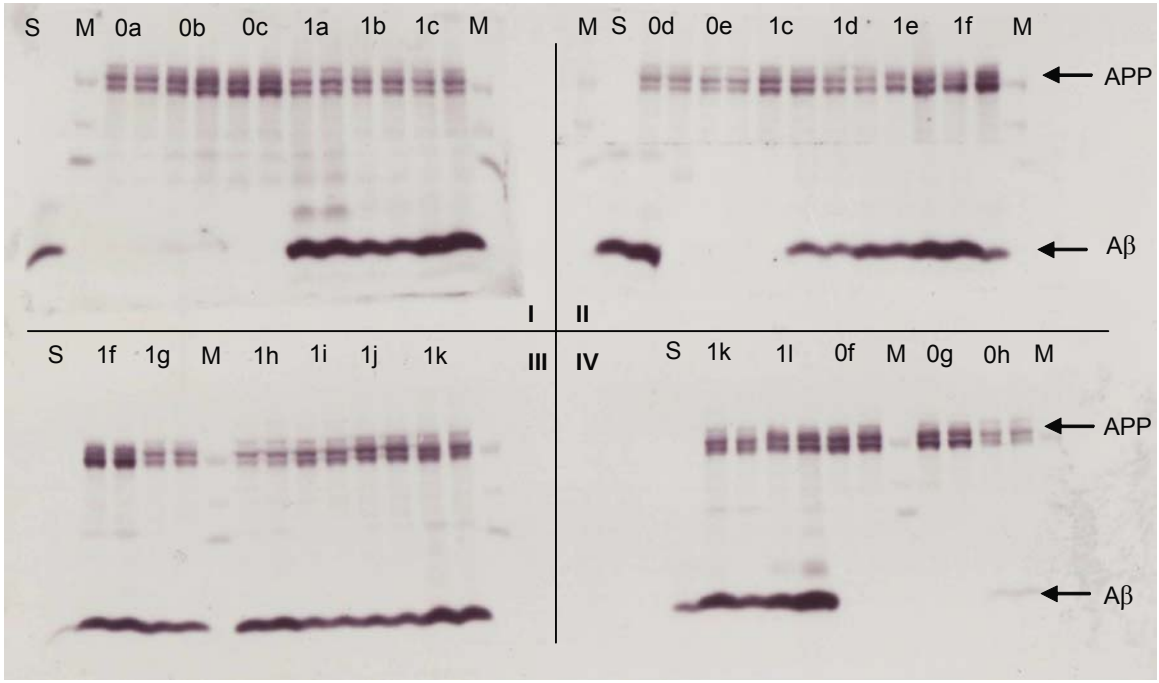

Fig. 18: Western Blot analysis for APP in brain samples

The upper band shows the amount of APP protein levels, whereas the lower band shows the amount of present Aβ protein levels in the examined brain samples. Each test item was analyzed in duplicates in adjacent lanes. 4 gels were run (I – IV) and inter-gel standardization was performed through repetition of test items on different gels. Note that controls are marked with 0, cases are marked with 1, M is the marker lane and S the used Aβ standard for check of the antibody. The test items are numbered alphabetically (controls 0a – 0 h, cases 1a – 1l).

A clear presence of Aβ was found in all subjects affected with AD (marked with 1 in Fig. 18). In contrast, for the controls (marked with 0 in Fig. 18) no Aβ above the detection limit of the assay could be determined at all.

Statistical analysis was performed for APP on basis of the revealed density of the bands for each sample. However, no significant association between the amount of present APP and the analyzed genotype in the brain samples was found.

5.1.4.3 Analyses of Aβ levels in brain samples

These same brain samples were then utilized to determine soluble levels of Aβ isoforms (Aβ38, 40, 42 and 40/42 ratio) using an ELISA. The results of the ANOVA analysis investigating the association of total Aβ, Aβ40 and Aβ42 with the determined genotypes are presented in the table below (Tab. 23). Significant results were stratified with post-hoc analyses (multiple testing using Tukey HSD = honest significant difference and LSD = least significant difference) to show which groups delivered the different results. SNPs # 35 (rs6516727) and 36 (rs2830099) showed a significant impact on total Aβ as well as on Aβ40.

For SNP # 35, C was associated with higher levels of total Aβ (TT (n=10) 250±321, TC (n=8) 310±382, CC (n=2) 1217±1431; p = 0.057, 0.046 (HSD), 0.020(LSD)) as well as with higher levels of Aβ40 (TT (n=10) 224±312, TC (n=8) 282±364, CC (n=2) 1169±1385; p = 0.057, 0.046 (HSD), 0.018(LSD)). Similarly, for SNP # 36, the C allele was associated with higher levels of total Aβ (GG (n=11) 249±305, GC (n=7) 320±412, CC (n=2) 1217±1431; p = 0.056, 0.047 (HSD), 0.019(LSD)) as well as with higher levels of Aβ40 (GG (n=11) 225±96, GC (n=7) 288±393, CC (n=2) 1169±1385; p = 0.053, 0.045 (HSD), 0.018(LSD)). SNP # 18 (rs2830019) yielded a significant p-value when testing association between genotype and Aβ42 level (t-test: CC (n=13) 16.6 ± 12.5, CT/TT (n=2) 39.5 ± 36.1; p = 0.041). No other significant associations were observed. For some of the analyzed SNPs no ANOVA analysis was possible, since only two groups were observed. In those cases results of a t-test are reported and results are marked in italic font.

Tab. 23: Results from the statistical analysis measuring the influence of sequence variation on total Aβ content.

#	SNP	p-values from ANOVA (association between genotype and total Aβ) / HSD / LSD	p-values from ANOVA (association between genotype and Aβ40) / HSD / LSD	p-values from ANOVA (association between genotype and Aβ42) / HSD / LSD
15	rs9978555	*0.542*	*0.540*	*0.574*
16	rs9941877	0.857	0.848	0.404
17	rs2830012	0.950	0.971	0.690
18	rs2830019	*0.422*	*0.413*	*0.041*
19	rs768039	0.799	0.812	0.422
20	rs3787644	0.799	0.812	0.422
21	rs2070655	0.550	0.565	0.733
22	rs3991	0.949	*0.417*	*0.117*
23	rs2830035	0.826	0.851	0.553
25	rs1041420	0.675	0.647	0.866
35	**rs6516727**	**0.057 / 0.049 / 0.020**	**0.053 / 0.046 (C-T) / 0.018 (C-T)**	0.220
36	**rs2830099**	**0.056 / 0.047 / 0.019**	**0.053 / 0.045 (G-C) / 0.018 (G-C)**	0.220

Results in *italic* letters: p-values from t-test, no ANOVA possible, 2 groups only

HSD = Tukey honest significant difference and LSD = least significant difference post-hoc testing

5.1.5 Functional Analyses for *APP* in Cerebrospinal Fluid

Additionally, a set of 85 cerebrospinal fluid (CSF) samples from AD patients was analyzed for their content of Aβ42. Due to the limited amount of these samples, only the 4 SNPs (SNPs # 16, 20, 35 and 36), which had shown significance in the complete Munich cohort, as well as the adjacent SNPs of the strongest association marker - SNP # 20 - (SNPs # 19 and 21) were selected for analyses.

In a set of 85 cerebrospinal fluid (CRF) samples from AD patients Aβ42 levels were determined using an ELISA. SNP # 36 revealed a significant association of homozygosity of the major allele (G) with elevated CSF Aβ42 levels (GG (n=40) 563.95 ± 276.7ng/L, GC/CC (n=42) 431.02 ± 191.1ng/L, p = 0.015); however, no significant associations were observed for the remaining SNPs. The results of the statistical analysis are presented in the table below.

Tab. 24: Results of statistical analysis of Aβ42 levels in CSF

#	SNP	p-values from ANOVA (association between genotype and Aβ42 level in CSF) / HSD / LSD	p-values from t-test (recessive for major allele)
16	rs9941877	0.990	0.923
19	rs768039	0.830	0.874
20	rs3787644	0.838	0.677
21	rs2070655	0.749	0.774
35	rs6516727	0.200	0.200
36	**rs2830099**	**0.048 / 0.061 / 0.024**	**0.015**

Note that lnAβ42 levels were used for evaluation so that normally distribution within the study cohort was given!

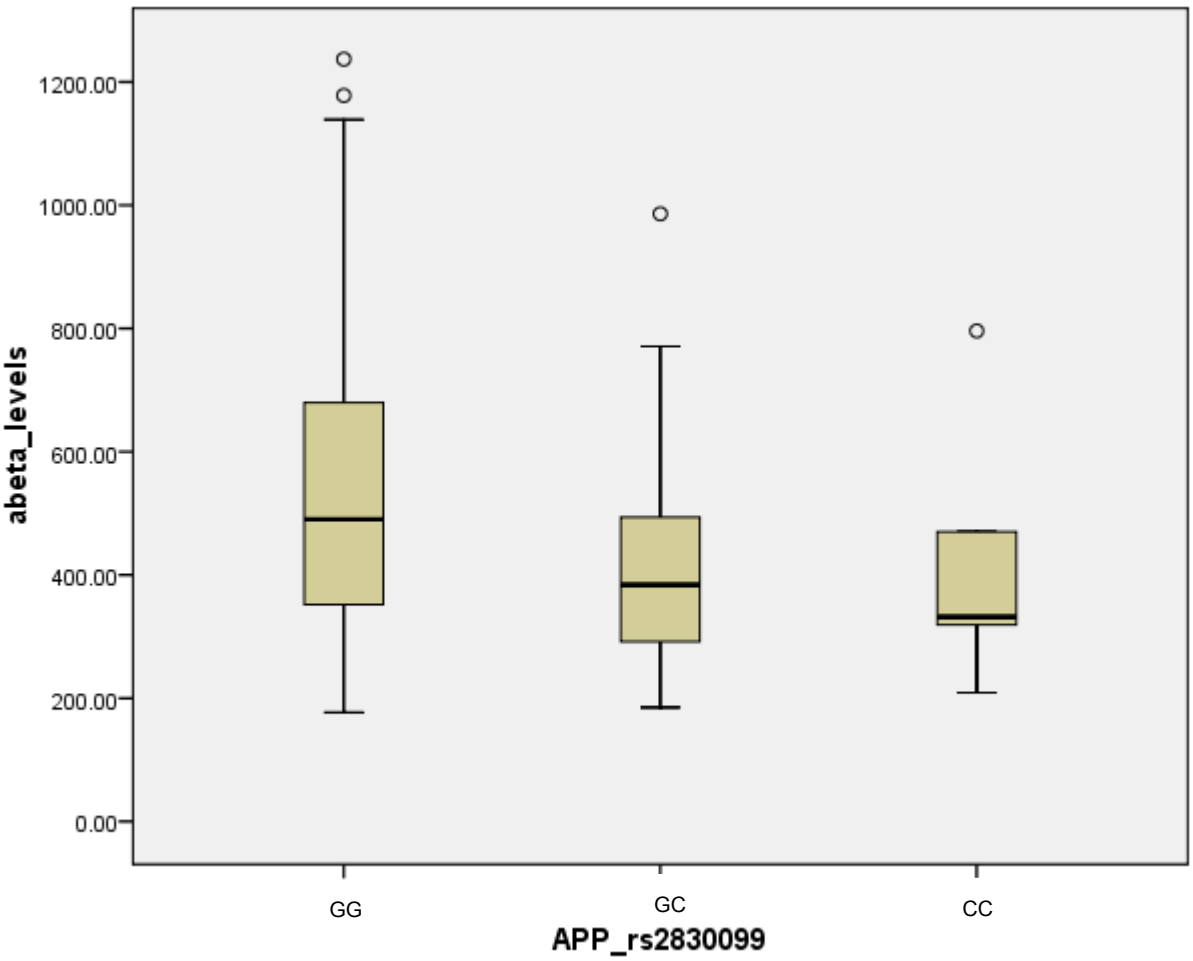

Fig. 19: Boxplot of Aβ42 levels in CSF for significant SNP rs2830099 (# 36)

The x-axis presents the genotypes, the y-axis the measured Aβ42 in ng/L CSF. Boxplot shows median (bar), the interquartile range (box = 50 % of values) and the range (lines = 100 % of values without outliers and extremes) of Aβ42 levels (○: Outliers i.e., cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box.) The box length is the interquartile range. Aβ42 levels were measured in ng/L CSF.

5.2 Analysis of SNPs from Candidate Genes Involved in APP Processing

Both *BACE1* and *ADAM10* represent, due to their involvement in APP processing, prime functional candidates for AD. Furthermore, the location of *BACE1* and *ADAM10* in the vicinity of suggestive AD linkage peaks at 11q25 (Blacker, Bertram et al. 2003) and 15q22 (Scott, Hauser et al. 2003), also make them positional candidates. Whilst *BACE1* has been the subject of study before, these reports have focused on one or two exon 5 polymorphisms (for meta-anlaysis see the AlzGene database (Bertram, McQueen et al. 2007)) with only one study utilizing a whole-gene approach (Todd, McKnight et al. 2008). On the other hand, polymorphisms in *ADAM10* have not received much attention in the literature (Prinzen, Muller et al. 2005). As such, this study aimed to comprehensively investigate the genetic role of both genes in AD.

5.2.1 Analysis of SNPs from *BACE1*

The *BACE1* gene is located in chromosomal region 11q23.2-q23.3 and contains 9 exons spanning 30.6 kilobases. The beta-site amyloid beta A4 precursor protein-cleaving enzyme 1 (BACE1) initiates the generation of beta-amyloid (Aβ) and therefore plays a major role during AD pathogenesis, as described before in detail (see Introduction, § 1.4.2.1).

The potential association of the *BACE1* gene in AD was thoroughly investigated by utilizing a fine mapping approach across the entire *BACE1* gene in a large German case-control series.

After several and thorough databank researches, 24 SNPs were selected for further study (Tab. 25). From these SNPs, 11 could be confirmed to be polymorphic in the Munich collective, achieved a sufficient genotype frequency (>80%) and showed no deviation from the HWE in the controls (Tab. 26 and Fig. 20). Incorporating results with insufficient genotype frequencies can lead to falsified distributions of the formerly matched cases and controls cohorts. Deviations from the HWE can be a sign of erroneous genotyping or a deficient population structure.

Tab. 25: Selected SNPs from the *BACE1* gene.

SNPs that yielded insufficient genotype frequencies (<80 %), significant deviations from HWE and minor allele frequencies <0.001 were not taken into account for the following statistical evaluations. SNPs passing all aforementioned criteria are highlighted in bold font. Alleles are reported in alphabetical order as found on http://snpper.chip.org.

SNP ID NCBI	SNP Position NCBI	Alleles	Role
rs7925044	**116653197**	**A/G**	**3' UTR**
rs12292027	**116654615**	**A/G**	**3' UTR**
rs2305821	116658630	C/T	3' UTR
rs3180616	116661628	A/T	Exon
rs7083	116661762	C/T	Exon
rs1047964	**116662103**	**C/G**	**Exon**
rs539765	116665557	A/G	Coding exon
rs490460	116668975	G/T	Intron (boundary)
rs638405	**116669034**	**C/G**	**Coding exon**
rs507805	**116672357**	**A/G**	**Intron**
rs548381	116676547	C/T	Intron
rs609332	**116680368**	**C/G**	**Intron**
rs522843	**116681144**	**A/G**	**Intron**
rs687740	**116682813**	**A/C**	**Intron**
rs473210	**116683010**	**A/G**	**Intron**
rs551662	**116684213**	**A/G**	**Intron**
rs676134	116685101	C/T	Intron
rs583161	116686131	C/T	Intron
rs656083	116687202	C/T	Intron
rs525493	**116687918**	**G/T**	**Intron**
rs2445939	116691283	A/T	Intron
rs3017609	116695997	A/T	Promoter
rs675228	116696661	A/G	Promoter
rs560564	116700838	A/C	Promoter

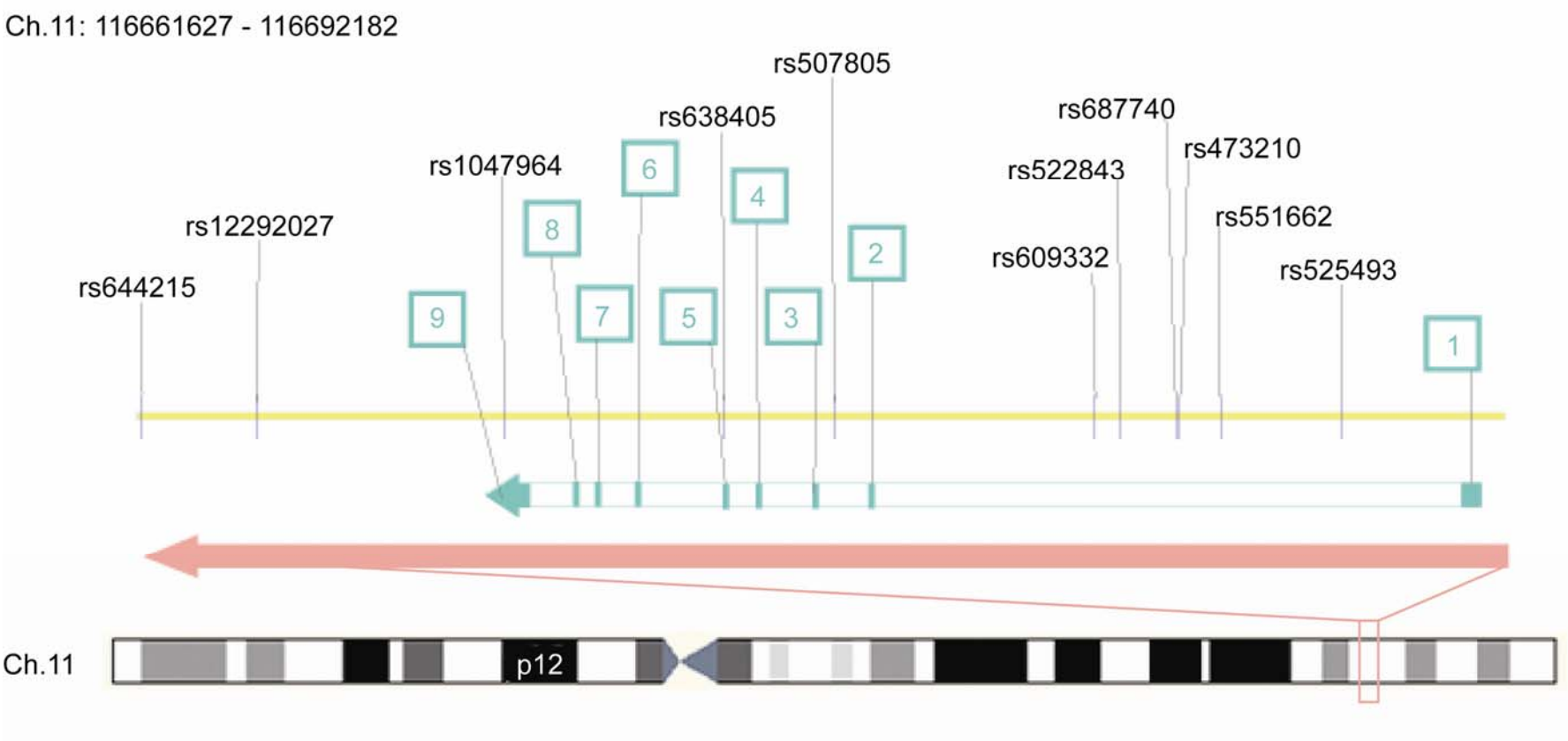

Fig. 20: Schematic of the *BACE1* gene.

The positions of the analyzed SNPs (shown with rs numbers) and exons in the *BACE1* gene are shown. Exons are marked with green bars on the forward strand from left to right. The arrow shows direction of transcription.

Single marker and haplotype associations were explored. Single marker analysis, within Haploview, of the 11 *BACE1* SNPs (Tab. 26) revealed no allelic associations with AD. Logistic regression analysis of genotypes with age and gender as covariates likewise revealed no significant associations (Tab. 27). The major allele frequencies (Tab. 26), as well as genotype frequencies (Tab. 27) were appropriately comparable in cases and controls and showed no clear differences. No single marker was associated with either increased risk, when stratified by age at onset of symptoms (see Appendix, § 7.3.2).

Tab. 26: Results from allelic tests for the *BACE1* gene.

Allelic associations were tested for significance using $\chi 2$-tests, all as implemented in Haploview version 4.2. All of the listed SNPs yielded genotype frequencies of at least 80 %, no significant deviation from HWE and minor allele frequencies were at least 0.001.

#	SNP	Associated allele	Major allele frequency MUC complete (cases, controls)	Allelic p-values
1	rs644215	A	0.634, 0.632	0.953
2	rs12292027	T	0.953, 0.948	0.662
3	rs1047964	C	0.936, 0.930	0.639
4	rs638405	C	0.595, 0.600.	0.859
5	rs507805	T	0.918, 0.905	0.370
6	rs609332	G	0.860, 0.848	0.525
7	rs522843	A	0.677, 0.656	0.407
8	rs687740	G	0.669, 0.691	0.391
9	rs473210	G	0.600, 0.583	0.515
10	rs551662	G	0.904, 0.906	0.879
11	rs525493	A	0.573, 0.551	0.395

Tab. 27: Results from genotypic tests for the *BACE1* gene.

Genotypic associations were tested for significance using a logistic regression recessive model [i.e. AA vs Aa,aa; where major allele is A] and are adjusted for age and gender (each SNP independently), all as implemented in SPSS.

SNP	Alleles (Major/Minor)	Genotype Frequency						
		Cases			Controls			p-values
		AA	Aa	aa	AA	Aa	aa	
rs644215	A/T	0.422	0.422	0.154	0.407	0.449	0.142	0.731
rs12292027	A/G	0.908	0.089	0.002	0.895	0.104	0.000	0.892
rs1047964	C/G	0.876	0.118	0.004	0.862	0.133	0.003	0.615
rs638405	C/G	0.350	0.489	0.160	0.353	0.493	0.153	0.826
rs507805	A/G	0.841	0.154	0.004	0.820	0.169	0.010	0.905
rs609332	C/G	0.737	0.243	0.018	0.730	0.235	0.034	0.748
rs522843	A/G	0.456	0.440	0.103	0.447	0.416	0.136	0.911
rs687740	A/C	0.464	0.408	0.126	0.472	0.437	0.090	0.740
rs473210	G/A	0.358	0.482	0.158	0.327	0.510	0.161	0.454
rs551662	A/G	0.824	0.159	0.016	0.819	0.173	0.007	0.911
rs525493	T/G	0.330	0.486	0.183	0.300	0.500	0.199	0.488

Thereafter, the LD structure for the *BACE1* gene was determined on basis of the 11 SNPs covering the complete genetic region. The average inter-marker distance was calculated to be 3.5 kb. Within the genotyped region 2 LD blocks were identified (Fig. 14). For each of them haplotypes were calculated as depicted in the table below (Tab. 28).

3 haplotypes were estimated within block 1, spanning SNPs # 1-2, 7 haplotypes within block 2, spanning SNPs # 4 – 10. None of the estimated haplotypes showed an association with AD, confirming the former findings.

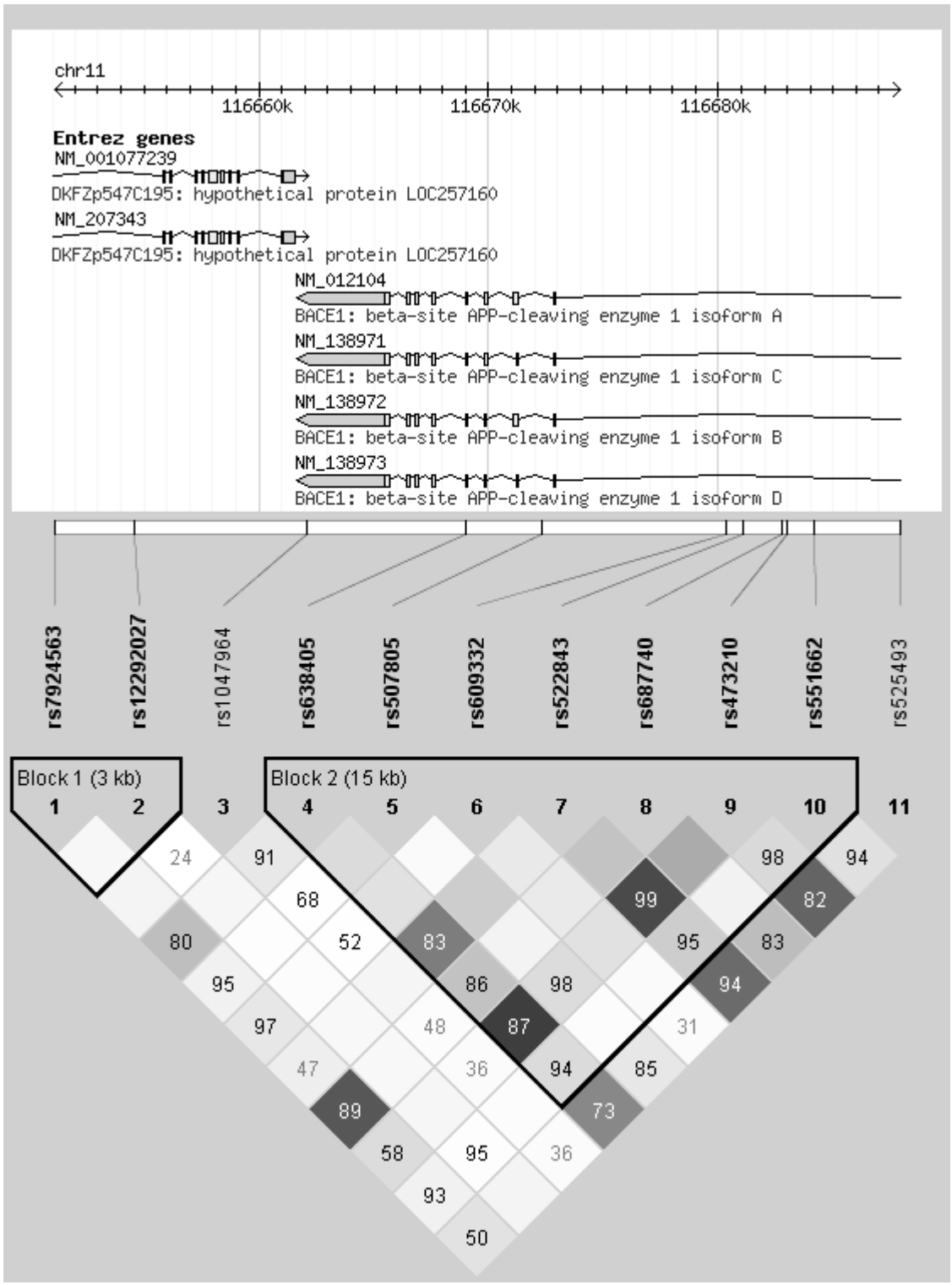

Fig. 21: LD structure of the the *BACE1* gene, including the haplotype block structure.

Values are used to represent D' whereas shades of grey were used to encode r^2 (D' values of 1.0 are never shown - the box is empty; black = high r^2 value, white = low r^2 value). The blocks followed the haplotype block definition by Gabriel et al., (Gabriel, Schaffner et al. 2002) as implemented within Haploview 4.2.

Tab. 28: Haplotype blocks and frequencies for *BACE1* in Munich collective

Haplotype ID	Sequence	Frequency (Case/Control)	P-values
Block 1			
1.1	AT	0.587, 0.580	0.801
1.2	TT	0.366, 0.368	0.954
1.3	AC	0.047, 0.052	0.655
Block 2			
2.1	GTGAGGA	0.315, 0.294	0.403
2.2	GTCATGA	0.141, 0.153	0.524
2.3	CTGGTAA	0.124, 0.117	0.667
2.4	GTGATGA	0.111, 0.112	0.934
2.5	CTGGTAG	0.091, 0.094	0.860
2.6	CCGGTAA	0.080, 0.095	0.301
2.7	CTGATAA	0.077, 0.074	0.808

In addition, haplotype analysis within *BACE1* also revealed no significant associations with AD. Results of the 2- and 3-marker sliding window approach to identify SNPs that contributed association signals are depicted in the figure below (Fig. 22). Detailed results from the 2- and 3-marker sliding window approach are depicted in the Appendix (§ 7.4.2). Haplotypes with frequencies below 5% were not taken into account, due to their unlikely occurrence.

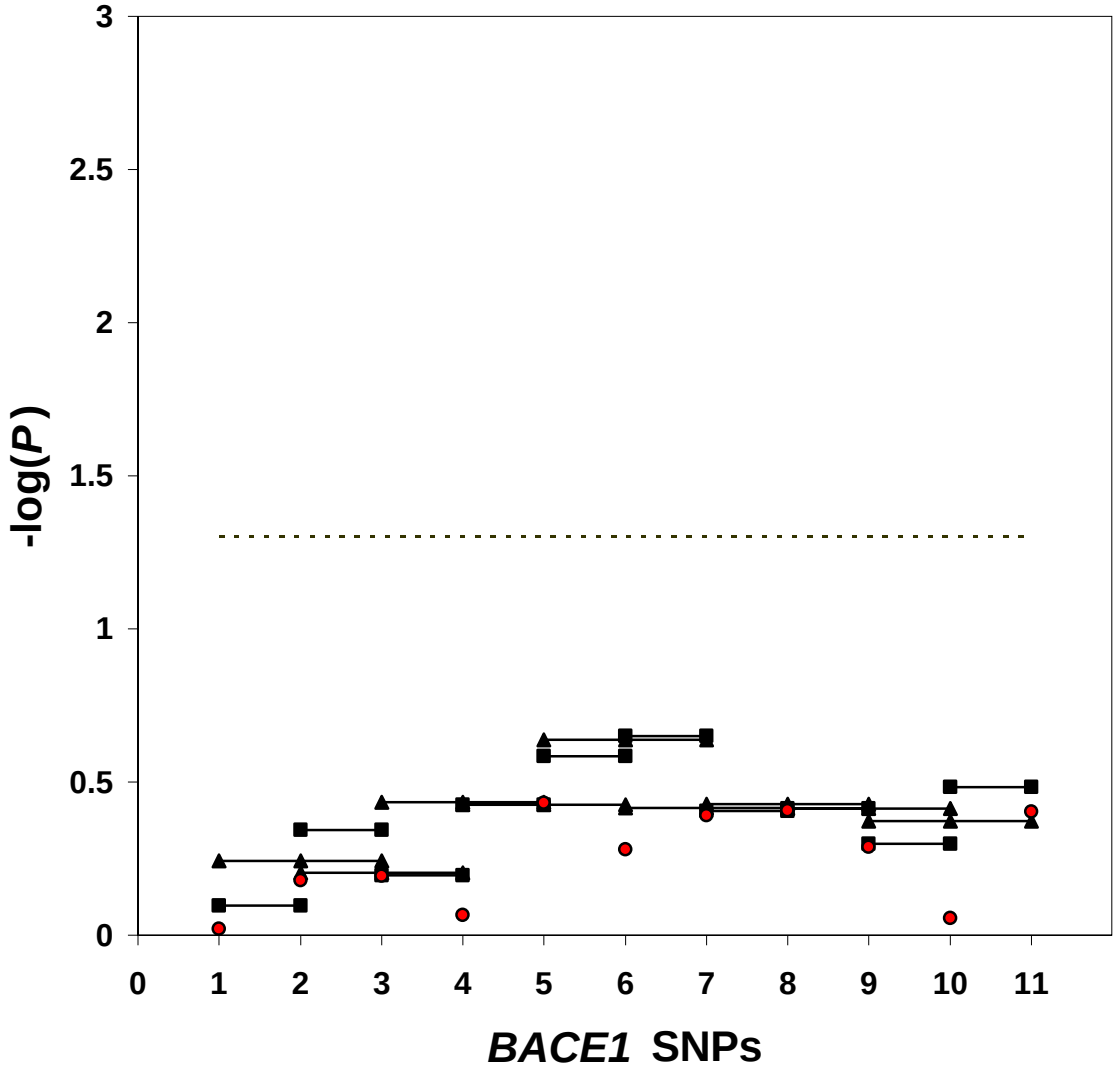

Fig. 22: Sliding window analysis of 2- and 3-marker haplotypes for *BACE1*

Red circles represent -log p values for single marker SNPs genotyped. Lines between solid squares represent the -log p value of 2-marker haplotypes. Lines between solid triangles mark -log p values for 3-marker haplotypes. All 2- and 3-marker haplotypes as calculated with Haploview. The dotted lines at a -log p value of approximately 1.3 demarcate the association threshold of p = 0.05. Numbers correspond to those assigned to SNPs by Haploview.

5.2.2 Analysis of SNPs from *ADAM10*

Central to AD pathogenesis is the amyloid cascade hypothesis (Hardy and Higgins 1992). An alternative cleavage pathway, involving α-secretase, precludes the formation of Aβ (Kojro and Fahrenholz 2005). ADAM10, a member of the ADAM (a disintegrin and metalloproteinase) family of proteinases and encoded by the *ADAM10* gene at 15q21-q23, has been shown to exhibit this α-secretase activity (Lammich, Kojro et al. 1999). The *ADAM10* gene contains 16 exons in 153.7 kilobases.

The potential association of the *ADAM10* gene in AD was thoroughly investigated by utilizing a fine mapping approach across the entire *ADAM10* gene in a large German case-control series.

After several and thorough databank researches, 41 SNPs were selected (Tab. 29). From these, 27 SNPs could be confirmed to be polymorph in the Munich collective, achieved a sufficient genotype frequency (>80%) and showed no deviation from the HWE in controls (Tab. 30 and Fig. 23).

Tab. 29: Selected SNPs from the *ADAM10* gene.

SNPs that yielded insufficient genotype frequencies (<80 %), significant deviations from HWE and minor allele frequencies <0.001 were not taken into account for the following statistical evaluations. SNPs passing all aforementioned criteria are highlighted in bold font. Alleles are reported in alphabetical order as found on http://snpper.chip.org.

SNP ID NCBI	SNP Position NCBI	Alleles	Role
rs12438487	**56666658**	**A/C**	**3' UTR**
rs1869135	**56669261**	**C/T**	**3' UTR**
rs3764196	56674302	G/T	3' UTR
rs7166076	**56679702**	**C/T**	**Intron**
rs1365772	56681600	C/T	Intron
rs7165035	**56687089**	**A/C**	**Intron**
rs1801973	56689928	G/T	Coding exon
rs2305421	**56690375**	**A/G**	**Intron**
rs8027151	56696272	A/G	Intron
rs8027618	56696273	A/G	Intron
rs12594872	**56700041**	**C/T**	**Intron**
rs1427282	**56701618**	**C/T**	**Intron**
rs8043406	56701924	A/G	Intron
rs972801	56708490	C/T	Intron

rs7163733	**56711611**	**C/T**	**Intron**
rs7163733	56711611	C/T	Intron
rs7174386	**56716605**	**C/T**	**Intron**
rs9302203	**56721161**	**A/T**	**Intron**
rs6494031	**56726560**	**C/T**	**Intron**
rs11071392	**56731962**	**G/T**	**Intron**
rs8026668	**56736062**	**A/T**	**Intron**
rs11071393	**56740318**	**C/G**	**Intron**
rs1427280	**56741612**	**A/G**	**Intron**
rs1427281	**56746565**	**C/T**	**Intron**
rs4775086	**56756584**	**A/G**	**Intron**
rs4775087	56762196	C/T	Intron
rs7167528	56763010	A/C	Intron
rs2052805	**56770279**	**A/G**	**Intron**
rs2657125	**56780693**	**C/G**	**Intron**
rs347117	**56788249**	**C/T**	**Intron**
rs2054096	56796135	A/T	Intron
rs4238331	**56799425**	**G/T**	**Intron**
rs12441313	**56802780**	**G/T**	**Intron**
rs6494038	**56807416**	**C/T**	**Intron**
rs12439231	**56812887**	**C/T**	**Intron**
rs7173286	56818300	A/T	Intron
rs383902	**56821466**	**C/T**	**Intron**
rs653765	**56829304**	**A/G**	**Exon**
rs593742	**56833066**	**A/G**	**Promoter**
rs681711	56837343	A/G	Promoter
rs694335	56838189	C/T	Promoter

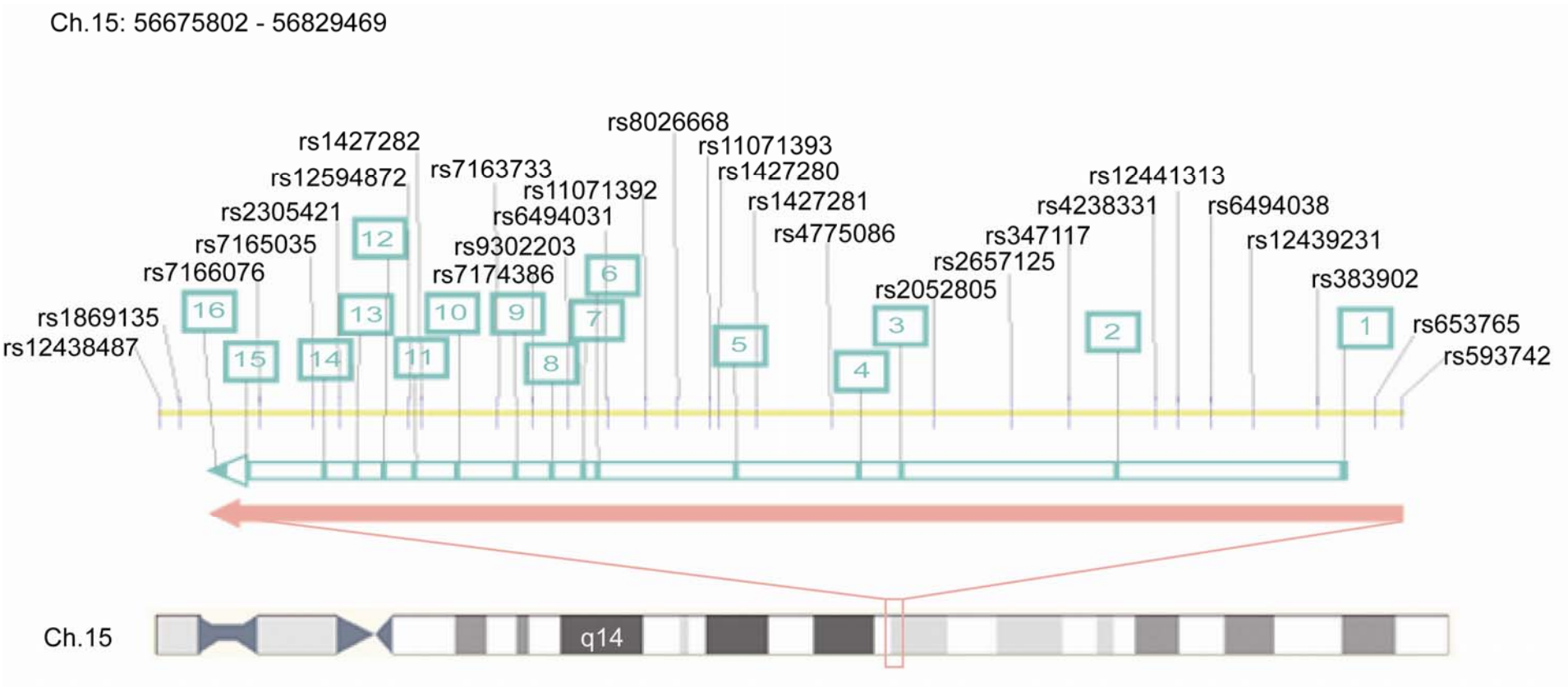

Fig. 23: Schematic of the *ADAM10* gene.

The positions of the analyzed SNPs (shown with rs numbers) and exons in the *ADAM10* gene are shown. Exons are marked with green bars on the forward strand from left to right. The arrow shows direction of transcription.

Single marker analysis, determined as implemented within Haploview, of the 27 *ADAM10* SNPs (Tab. 30) revealed no allelic associations with AD. Logistic regression analysis of genotypes likewise revealed no significant associations (Tab. 31). The major allele frequencies (Tab. 30), as well as genotype frequencies (Tab. 31) were similar in cases and controls and showed no notable differences. None of the single markers was associated with increased risk for AD, when stratified by age at onset of symptoms (see Appendix, § 7.3.3).

Tab. 30: Results from allelic tests for the *ADAM10* gene.

Allelic associations were tested for significance using $\chi 2$-tests, all as implemented in Haploview version 4.2. All of the listed SNPs yielded genotype frequencies of at least 80 %, no significant deviation from HWE and minor allele frequencies had to be at least 0.001.

#	SNP	Associated allele	Major allele frequency MUC complete (cases, controls)	Allelic p-values
1	rs12438487	T	0.888, 0.888	0.991
2	rs1869135	G	0.782, 0.784	0.935
3	rs7166076	G	0.881, 0.890	0.623
4	rs7165035	T	0.882, 0.897	0.376
5	rs2305421	C	0.881, 0.889	0.618
6	rs12594872	G	0.628, 0.630	0.933
7	rs1427282	T	0.669, 0.670	0.969
8	rs7163733	A	0.886, 0.894	0.650
9	rs7174386	G	0.877, 0.888	0.555
10	rs9302203	T	0.881, 0.886	0.750
11	rs6494031	A	0.894, 0.906	0.452
12	rs11071392	C	0.876, 0.889	0.441
13	rs8026668	A	0.890, 0.889	0.964
14	rs11071393	G	0.877, 0.889	0.485
15	rs1427280	C	0.893, 0.883	0.543
16	rs1427281	G	0.755, 0.762	0.758
17	rs4775086	T	0.767, 0.774	0.766
18	rs2052805	C	0.891, 0.893	0.911
19	rs2657125	C	0.856, 0.863	0.695
20	rs347117	A	0.729, 0.731	0.945
21	rs4238331	A	0.720, 0.733	0.612
22	rs12441313	A	0.888, 0.898	0.526
23	rs6494038	A	0.891, 0.895	0.828
24	rs12439231	A	0.884, 0.898	0.413
25	rs383902	A	0.703, 0.705	0.928
26	rs653765	G	0.725, 0.737	0.604
27	rs593742	C	0.711, 0.723	0.601

Tab. 31: Results from genotypic tests for the ADAM10 gene.

Genotypic associations were tested for significance using a logistic regression recessive model [i.e. AA vs Aa,aa; where major allele is A] and are adjusted for age and gender (each SNP independently) all as implemented in SPSS

SNP	Alleles (Major/Minor)	Genotype Frequency						
		Cases			Controls			p-values
		AA	Aa	aa	AA	Aa	aa	
rs12438487	C/A	0.800	0.176	0.024	0.794	0.188	0.018	0.899
rs1869135	T/C	0.620	0.324	0.056	0.621	0.326	0.053	0.864
rs7166076	T/C	0.784	0.195	0.021	0.797	0.185	0.017	0.622
rs7165035	C/A	0.790	0.184	0.026	0.813	0.169	0.018	0.421
rs2305421	A/G	0.783	0.196	0.021	0.796	0.186	0.018	0.606
rs12594872	T/C	0.393	0.469	0.138	0.391	0.478	0.131	0.889
rs1427282	C/T	0.451	0.435	0.114	0.434	0.472	0.094	0.777
rs7163733	C/T	0.794	0.186	0.021	0.806	0.177	0.017	0.673
rs7174386	T/C	0.778	0.200	0.023	0.792	0.190	0.017	0.597
rs9302203	A/T	0.783	0.196	0.021	0.790	0.192	0.017	0.791
rs6494031	C/T	0.807	0.174	0.019	0.831	0.151	0.018	0.427
rs11071392	T/G	0.774	0.202	0.023	0.796	0.187	0.018	0.487
rs8026668	A/T	0.805	0.171	0.024	0.800	0.179	0.021	0.916
rs11071393	C/G	0.777	0.200	0.023	0.795	0.188	0.017	0.516
rs1427280	G/A	0.816	0.156	0.028	0.780	0.206	0.014	0.271
rs1427281	T/C	0.584	0.342	0.074	0.585	0.356	0.060	0.898
rs4775086	G/A	0.598	0.340	0.063	0.596	0.354	0.049	0.960
rs2052805	A/G	0.801	0.180	0.019	0.807	0.172	0.021	0.830
rs2657125	C/G	0.734	0.243	0.023	0.743	0.240	0.017	0.618
rs347117	C/T	0.576	0.307	0.117	0.542	0.378	0.080	0.381
rs4238331	G/T	0.543	0.355	0.102	0.559	0.346	0.094	0.640
rs12441313	G/T	0.794	0.187	0.019	0.814	0.168	0.018	0.562
rs6494038	C/T	0.803	0.176	0.021	0.807	0.175	0.018	0.869
rs12439231	C/T	0.789	0.190	0.021	0.813	0.170	0.017	0.463
rs383902	C/T	0.518	0.369	0.112	0.511	0.388	0.101	0.838
rs653765	A/G	0.544	0.362	0.094	0.536	0.401	0.062	0.848
rs593742	A/G	0.515	0.391	0.094	0.529	0.388	0.083	0.735

Finally, the LD structure for the *ADAM10* gene was determined on basis of the 27 SNPs, covering the complete genetic region. The average inter-marker distance was calculated to be 6.4 kb. Within the genotyped region 2 LD blocks were identified on basis of their D' and r^2 values (Fig. 24). For each of them, haplotypes were calculated as depicted in the table below (Tab. 32). The LD blocks span the entire gene, block 1 from SNPs # 1-24, block 2 from SNPs # 25-27. Since D' values were high throughout all SNPs (majority values

>0.9), determination of one block spanning the whole genome would be possible as well. None of the estimated haplotypes showed an association with AD.

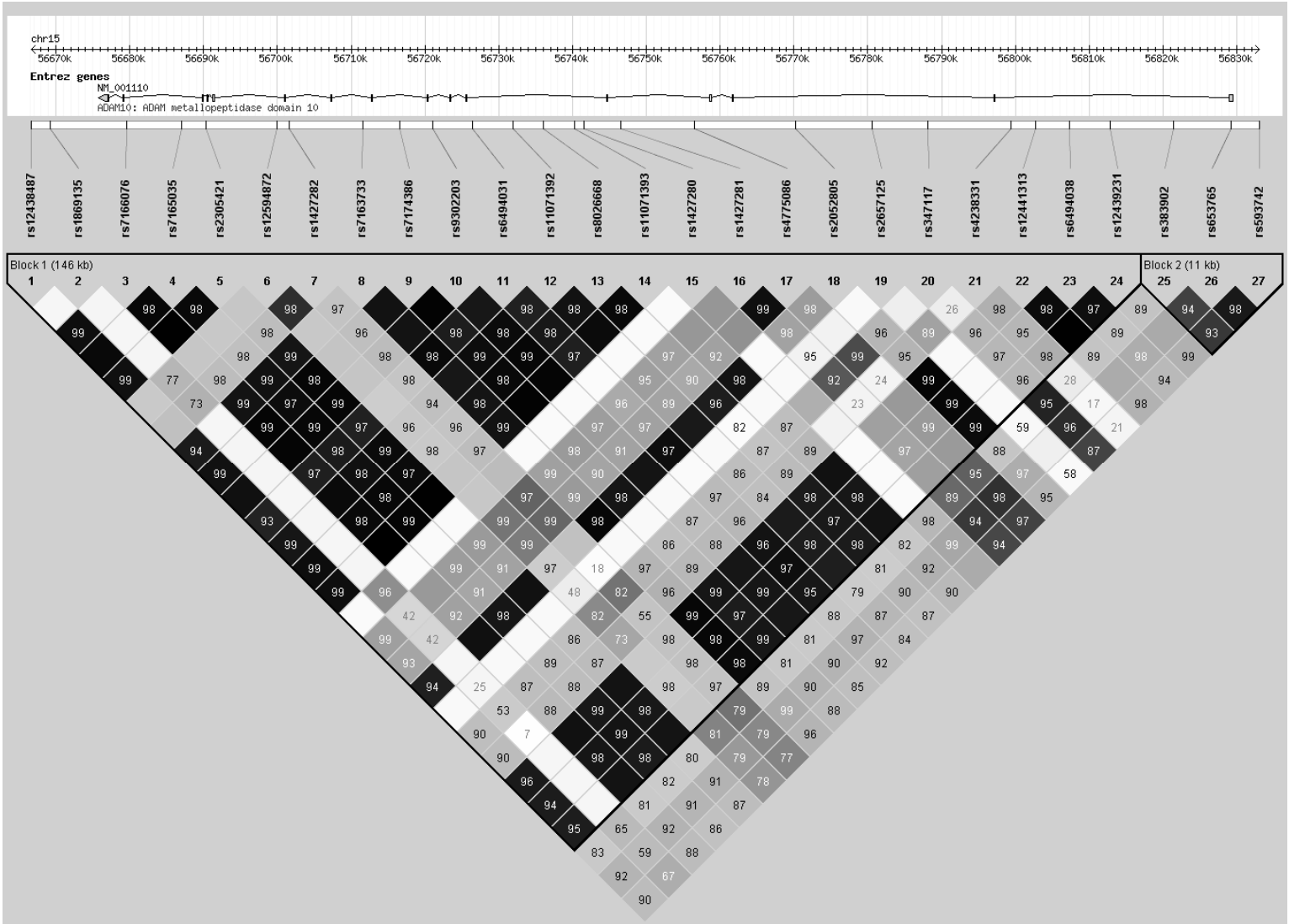

Fig. 24: LD structure of the *ADAM10* gene, including the haplotype block structure.

Numerical values are used to represent D' whereas colors were used to encode r^2 (D' values of 1.0 are never shown - the box is empty; black = high r^2 value, white = low r^2 value). The blocks followed the haplotype block definition by Gabriel et al., (Gabriel, Schaffner et al. 2002) as implemented within Haploview 4.2.

Tab. 32: Haplotype blocks and frequencies for *ADAM10* in the Munich collective

Haplotype ID	Sequence	Frequency (Case/Control)	P-values
Block 1			
1.1	GAAGTACGAAGAACCACTGGCCGG	0.534, 0.565	0.254
1.2	GGAGTGTGAAGAACTGTTGACCGG	0.115, 0.126	0.532
1.3	TAGTCGTAGTACTGCGTCGAAAAA	0.099, 0.098	0.962
1.4	GGAGTGTGAAGAACCACTCGACGG	0.061, 0.059	0.853
Block 2			
2.1	GAT	0.680, 0.677	0.903
2.2	AGC	0.266, 0.245	0.356

A 2- and 3-marker sliding window approach to identify SNPs that contributed association signals neither yielded any significant results (Fig. 25). Detailed results from the 2- and 3-marker sliding window approach are depicted in the Appendix (§ 7.4.3). Haplotypes with frequencies below 5% were not taken into account, due to their unlikely occurrence.

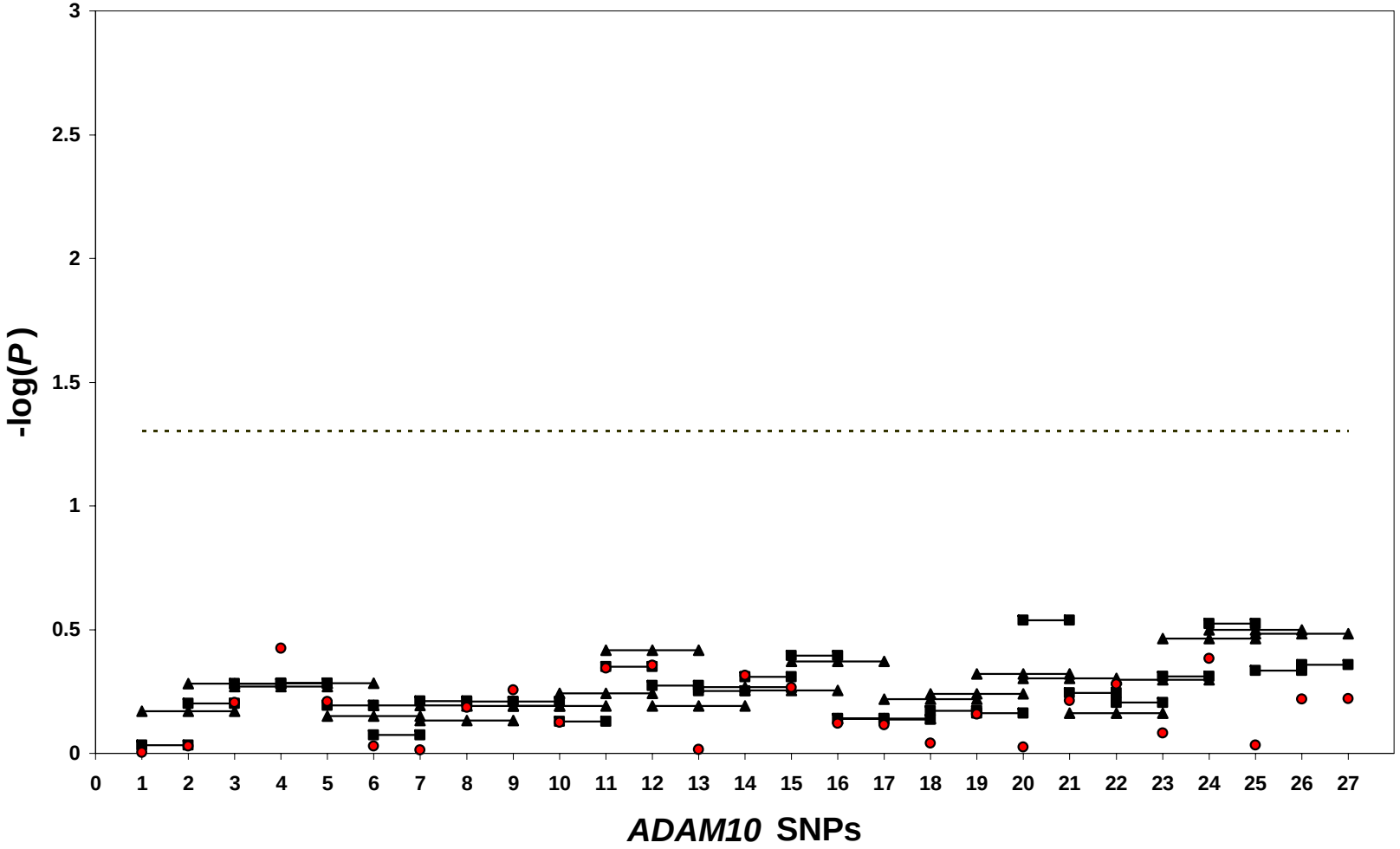

Fig. 25: Sliding window analysis of 2- and 3-marker haplotypes for *ADAM10*

Red circles represent -log p values for single marker SNPs genotyped. Lines between solid squares represent the -log p value of 2-marker haplotypes. Lines between solid triangles mark -log p values for 3-marker haplotypes. All 2- and 3-marker haplotypes as calculated with Haploview. The dotted lines at a -log p value of approximately 1.3 demarcate the threshold of p = 0.05. Numbers correspond to those assigned to SNPs by Haploview.

5.3 Analysis of SNPs from Candidate Genes Involved in Lipid Metabolism

Additionally, genes involved in the lipid metabolism, just like the major genetic risk factor for Alzheimer's disease (AD), apolipoprotein E (ApoE) were taken into consideration during selection of candidate genes.

5.3.1 Analysis of SNPs from *ABCA1*

The *ABCA1* gene is located on chromosome 9 (9q31.1), spans 149 kb and contains 50 exons. Since the gene is present approximately 5 Mb distal from previously identified loci linked with AD it is a potential positional candidate gene. Involvement into lipid metabolism also makes it a functional candidate. Up to now only one study used an approximate fine-mapping approach - still only including 19 SPNs, in a Chinese cohort (Chu, Li et al. 2007). Therefore, a whole fine mapping approach of *ABCA1* is of utmost importance to verify the former findings and to get results for the entire gene locus. Significant results underwent replication in a second large independent case-control series, as well as in an independent sib-pairs cohort. Additionally, associated SNPs underwent further analyses to elucidate their association with altered ABCA1 gene expression.

5.3.1.1 Genotyping of SNPs from *ABCA1*

After several databank researches, 48 SNPs were selected (Tab. 33). From these 30 could be confirmed to be polymorph in the Munich collective, achieved a sufficient genotype frequency (>80%) and showed no deviation from the HWE in controls (Tab. 34 and Fig. 26).

Tab. 33: Selected SNPs from the *ABCA1* gene.

SNPs that yielded insufficient genotype frequencies (<80 %), significant deviations from HWE and minor allele frequencies <0.001 were not taken into account for the following statistical evaluations. SNPs passing all aforementioned criteria are highlighted in bold font. Alleles are reported in alphabetical order as found on http://snpper.chip.org.

SNP ID NCBI	SNP Position NCBI	Alleles	Role
rs1858555	106552252	A/G	Intron
rs2515606	**106576716**	**C/T**	**3' UTR**
rs10761085	106581889	G/T	3' UTR
rs363717	106584521	A/G	Exon
rs1331924	**106587443**	**C/G**	**Intron (boundary)**
rs2274872	106589328	C/T	Intron
rs2066720	**106593890**	**A/G**	**Intron**
rs2777799	**106598880**	**A/G**	**Intron**
rs2230808	**106602625**	**A/G**	**Coding exon**
rs1997618	106604190	C/T	Coding exon
rs2066716	**106608526**	**A/G**	**Coding exon**
rs2297409	106616067	A/G	Intron
rs2297401	**106619293**	**A/C**	**Intron**
rs3818689	**106624837**	**C/G**	**Intron (boundary)**
rs4149313	**106626574**	**A/G**	**Coding exon**
rs2066718	**106629076**	**A/G**	**Coding exon**
rs13306068	106633803	A/G	Coding exon
rs2297398	**106638705**	**G/T**	**Intron**
rs2487062	**106643260**	**C/G**	**Intron**
rs914544	**106647033**	**C/T**	**Intron**
rs1175293	**106653704**	**C/T**	**Intron**
rs2472445	**106659171**	**G/T**	**Intron**
rs2230806	**106660688**	**A/G**	**Coding exon**
rs2230805	**106663850**	**A/G**	**Coding exon**
rs13301006	**106669157**	**C/T**	**Intron**
rs2000069	**106675690**	**C/T**	**Intron**
rs1999429	**106680607**	**A/C**	**Intron**
rs2275542	**106684901**	**C/T**	**Intron**
rs7043081	106689432	A/T	Intron
rs2275545	106691347	A/G	Intron (boundary)
rs3847305	**106697074**	**C/G**	**Intron**
rs2575875	**106702315**	**A/G**	**Intron**
rs3758294	**106704636**	**C/T**	**Intron**
rs7030810	106710980	C/T	Intron
rs1175453	106716527	A/G	Intron
rs2515616	106721816	C/T	Intron
rs2515611	106727317	C/T	Intron
rs1800977	106730271	C/T	Promoter
rs2487042	**106734343**	**A/G**	**Promoter**
rs2472490	106738148	A/G	Promoter
rs4743765	106739189	A/T	Promoter
rs2487035	**106741245**	**A/G**	**Intergenic**
rs2487030	106746009	A/G	Intergenic
rs7029538	106750844	C/T	Intergenic
rs2472516	**106756217**	**C/T**	**Intergenic**
rs4397467	**106762399**	**A/G**	**Intergenic**
rs4743776	**106767548**	**A/G**	**Intergenic**
rs737623	**106774926**	**A/G**	**Intergenic**

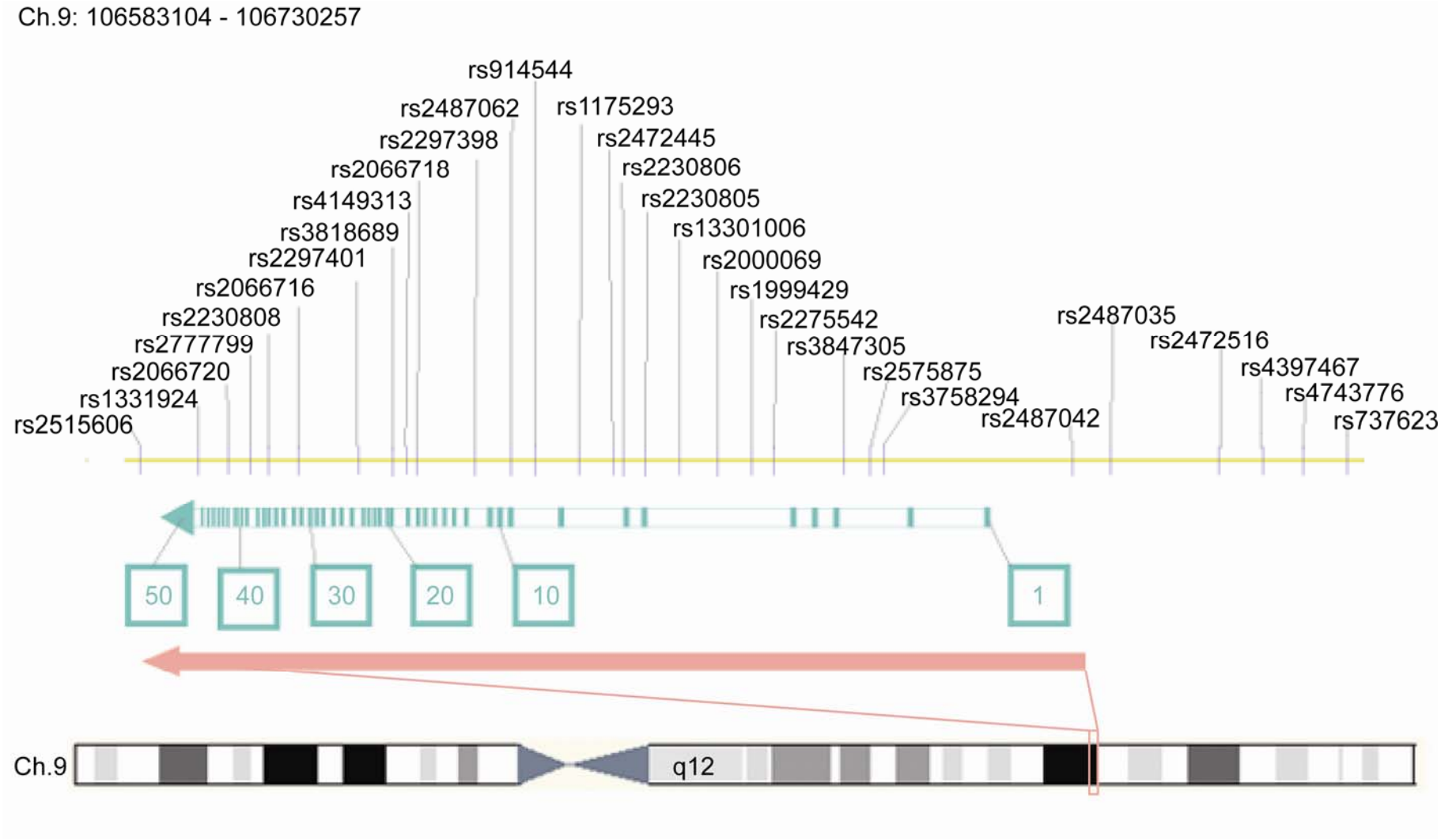

Fig. 26: Schematic of the *ABCA1* gene.

The positions of the analyzed SNPs (shown with rs numbers) and exons in the *ABCA1* gene are shown. Exons are marked with green bars on the forward strand from left to right. The arrow shows direction of transcription.

The subsequent allelic tests yielded no significant association between any SNP and AD in the complete Munich collective. However, a detailed analyses stratified by age at onset yielded significant p-values for two SNPs (SNP # 25, rs2487042, p-value=0.028 and SNP # 28, rs4397467, p-value=0.046) in subjects with EOAD. No significance was detected in subjects with LOAD. Stratification by presence or absence of the *ApoE* ε4 allele yielded another significant SNP (# 8, rs3818689, p-value=0.037). Allelic association tests yielded no further significant p-values.

Results from allelic tests of all SNPs performed for the Munich collective are depicted in Tab. 17.

Subsequently, genotyping analyses were performed for the same 30 SNPs using logistic regression with gender and age at onset as covariates. In the complete Munich collective 2 SNPs could be shown to be associated with AD. An association signal was found for the intronic SNP rs2066720 (# 3) with a p-value of 0.047. The major allele (G) was associated with a higher risk for AD (OR = 2.75). For the intronic SNP # 1 (rs2515606) a borderline value of 0.05 was observed. The C allele was associated with a higher risk for AD (OR = 1.53). In the genotypic approach none of the formerly associated SNPs could be confirmed in subjects with EOAD or LOAD, but stratification for EOAD identified an additional association signal for SNP # 26. Results from genotypic tests of all SNPs performed for the Munich collective are depicted in Tab. 35. Stratification for presence of the *ApoE* ε4 allele was also performed for the genotypic approach. SNPs # 1 was confirmed for the non *ApoE* ε4 sub-collective an equal p-value as for the whole collective. One additional significant signal was found for SNP # 28 (rs4397467) with a p- value of 0.015 in the non *ApoE* ε4 sub-collective. The association of SNP # 3 with AD was also confirmed for the *ApoE* ε4 sub-collective with a p-value of 0.045, but this time the A allele was associated with a higher risk. Results after stratification for presence of *ApoE* ε4 allele are presented in the Appendix (§ 7.3.4, Tab. 54).

Tab. 34: Results from allelic tests for *ABCA1* gene.

Allelic associations were tested for significance using $\chi 2$-tests, all as implemented in Haploview version 4.2. The statistical analysis were carried out for the whole collective as well as for sub-collectives divided by age/onset (EOAD ≤ 65 years, LOAD > 65 years) and presence of at least one ApoE4allele (ApoE4 = at least one ApoE4 allele, nonApoE4 = no ApoE4 allele) for cases and controls. All shown SNPs yielded genotype frequencies of at least 80 %, no significant deviation from HWE and minor allele frequencies had to be at least 0.001. Significant results are highlighted in bold font.

#	SNP	Allelic test results						
		Associated allele	Major allele frequency MUC complete (cases, controls)	Allelic p-values				
				MUC complete (N=728)	EOAD subcollective (N=281)	LOAD subcollective (N=444)	*ApoE* ε4 subcollective (N = 295)	non *ApoE* ε4 subcollective (N = 433)
1	rs2515606	C	0.573, 0.624	0.058	0.129	0.292	0.956	0.063
2	rs1331924	G	0.885, 0.864	0.239	0.939	0.216	0.247	0.435
3	rs2066720	G	0.881, 0.869	0.533	0.986	0.448	0.065	0.453
4	rs2777799	C	0.871, 0.884	0.457	0.296	0.814	0.973	0.788
5	rs2230808	A	0.730, 0.743	0.578	0.184	0.735	0.072	0.803
6	rs2066716	G	0.919, 0.910	0.528	0.949	0.308	0.303	0.941
7	rs2297401	T	0.951, 0.943	0.528	0.764	0.455	0.459	0.982
8	**rs3818689**	G	0.947, 0.962	0.190	0.065	0.912	**0.037**	0.533
9	rs4149313	G	0.872, 0.893	0.224	0.113	0.914	0.852	0.131
10	rs2066718	A	0.972, 0.973	0.851	0.311	0.528	0.887	0.518
11	rs2297398	C	0.928, 0.940	0.415	0.235	0.929	0.343	0.229
12	rs2487062	G	0.892, 0.901	0.620	0.619	0.393	0.691	0.974
13	rs914544	G	0.952, 0.958	0.578	0.926	0.544	0.285	0.270
14	rs1175293	A	0.930, 0.925	0.725	0.475	0.241	0.321	0.945
15	rs2472445	T	0.855, 0.855	0.998	0.652	0.851	0.667	0.916
16	rs2230806	A	0.691, 0.700	0.722	0.833	0.762	0.625	0.632

17	rs2230805	A	0.715, 0.729	0.570	0.755	0.923	0.560	0.455
18	rs13301006	A	0.838, 0.868	0.125	0.140	0.506	0.761	0.373
19	rs2000069	A	0.537, 0.556	0.474	0.326	0.892	0.684	0.913
20	rs1999429	C	0.984, 0.972	0.126	0.727	0.095	0.631	0.401
21	rs2275542	G	0.678, 0.667	0.645	0.507	0.853	0.259	0.543
22	rs3847305	G	0.870, 0.873	0.879	0.710	0.970	0.950	0.996
23	rs2575875	T	0.616, 0.617	0.963	0.876	0.943	0.297	0.891
24	rs3758294	G	0.777, 0.790	0.562	0.706	0.491	0.302	0.778
25	**rs2487042**	A	0.772, 0.796	0.283	**0.028**	0.795	0.865	0.310
26	rs2487035	T	0.657, 0.655	0.956	0.073	0.289	0.183	0.424
27	rs2472516	G	0.607, 0.615	0.767	0.394	0.858	0.135	0.174
28	**rs4397467**	G	0.681, 0.719	0.127	**0.046**	0.370	0.633	0.036
29	rs4743776	G	0.703, 0.708	0.833	0.141	0.351	0.251	0.584
30	rs737623	G	0.576, 0.563	0.644	0.220	0.992	0.448	0.246

Tab. 35: Results from genotypic tests for *ABCA1* gene.

Genotypic associations were tested for significance using logistic regression, all as implemented in STATA/SE 9.2. The statistical analysis were carried out for the whole collective as well as for sub-collectives divided by age/onset (EOAD ≤ 65 years, LOAD > 65 years) and presence of at least one ApoE4allele (ApoE4 = at least one ApoE4 allele, nonApoE4 = no ApoE4 allele) for cases and controls. Gender and age/onset were taken into account as covariates. Significant results are highlighted in bold font. Note that SNP # 5 could not be evaluated, due to no occurrence of the minor allele in control group.

#	SNP	Alleles (major/ minor)	Genotypic test results					
			Genotype frequencies MUC complete (cases, controls)			AD affection genotypic p-values for major allele unless otherwise noted (associated allele); OR (95%CI) with covariates (sex and onset)		
			AA	Aa	aa	MUC complete (N=728)	EOAD subcollective (N=281)	LOAD subcollective (N=444)
1	**rs2515606**	T/C	0.337, 0.380	0.473, 0.488	0.191, 0.133	**0.050 (C); 1.53 (0.9-2.3)**	0.365	0.219
2	rs1331924	G/C	0.791, 0.754	0.192, 0.222	0.019, 0.025	0.310	0.561	0.549
3	**rs2066720**	G/A	0.778, 0.782	0.207, 0.175	0.016, 0.044	**0.047 (G); 2.75 (1.01-7.5)**	0.647	0.841
4	rs2777799	T/C	0.760, 0.786	0.222, 0.197	0.019, 0.018	0.357	0.372	0.599
5	rs2230808	G/A	0.533, 0.560	0.397, 0.368	0.072, 0.074	0.444	0.290	0.952
6	rs2066716	G/A	0.849, 0.827	0.143, 0.167	0.010, 0.008	0.467	0.602	0.428
7	rs2297401	T/G	0.902, 0.890	0.099, 0.107	0.000, 0.004	0.671	0.789	0.712
8	rs3818689	C/G	0.897, 0.928	0.102, 0.069	0.003, 0.004	0.184	0.158	0.727
9	rs4149313	A/G	0.761, 0.819	0.223, 0.151	0.017, 0.032	0.087	0.089	0.368
10	rs2066718	G/A	0.944, 0.951	0.057, 0.047	0.000, 0.004	0.682	0.208	0.950
11	rs2297398	A/C	0.862, 0.894	0.134, 0.093	0.005, 0.015	0.254	0.213	0.455
12	rs2487062	C/G	0.785, 0.802	0.216, 0.199	0.000, 0.000	0.528	0.599	0.535
13	rs914544	A/G	0.906, 0.923	0.092, 0.071	0.003, 0.008	0.440	0.582	0.313
14	rs1175293	A/G	0.874, 0.864	0.113, 0.123	0.014, 0.014	0.608	0.942	0.396
15	rs2472445	G/T	0.725, 0.721	0.263, 0.270	0.014, 0.011	0.999	0.821	0.961
16	rs2230806	G/A	0.481, 0.483	0.422, 0.436	0.099, 0.083	0.973	0.549	0.705

17	rs2230805	G/A	0.518, 0.525	0.398, 0.410	0.086, 0.067	0.887	0.810	0.801
18	rs13301006	G/A	0.700, 0.761	0.277, 0.215	0.024, 0.025	0.096	0.105	0.899
19	rs2000069	G/A	0.292, 0.316	0.491, 0.481	0.219, 0.204	0.551	0.401	0.446
20	rs1999429	C/A	0.968, 0.944	0.033, 0.057	0.000, 0.000	0.137	0.736	0.160
21	rs2275542	G/A	0.454, 0.447	0.451, 0.440	0.097, 0.114	0.821	0.832	0.766
22	rs3847305	C/G	0.755, 0.767	0.232, 0.213	0.015, 0.022	0.774	0.642	0.671
23	rs2575875	C/T	0.364, 0.388	0.505, 0.460	0.132, 0.154	0.584	0.580	0.786
24	rs3758294	A/G	0.634, 0.645	0.288, 0.292	0.079, 0.065	0.695	0.987	0.466
25	rs2487042	G/A	0.608, 0.629	0.329, 0.336	0.064, 0.036	0.590	0.061	0.389
26	**rs2487035**	T/C	0.445, 0.456	0.424, 0.400	0.132, 0.145	0.836	**0.033 (T); 2.47 (1.1-5.7)**	0.173
27	rs2472516	A/G	0.371, 0.388	0.472, 0.456	0.158, 0.158	0.662	0.449	0.817
28	rs4397467	A/G	0.472, 0.513	0.419, 0.414	0.110, 0.075	0.280	0.352	0.443
29	rs4743776	A/G	0.503, 0.502	0.402, 0.414	0.096, 0.085	0.774	0.201	0.400
30	rs737623	G/A	0.354, 0.321	0.447, 0.486	0.200, 0.194	0.239	0.262	0.463

The 30 SNPs covering the complete genetic region of the *ABCA1* gene (149 kb, average inter-marker distance of 6.8 kb) determined the linkage disequilibrium (LD) structure, estimated by D' and r^2 and explored haplotype associations as implemented in Haploview. Within the genotyped region 8 LD blocks were identified on basis of D' and r^2 values (Fig. 27). For each of them, haplotypes were calculated as depicted in the table below (Tab. 43). The 2 significant markers identified in the Munich collective before were found outside of any block. SNP # 3 showed a slight correlation with the direct neighbor SNP # 2 ($r^2 = 0.01$, D' = 0.88). No other correlation was found for SNPs # 1 or 3. None of the estimated haplotypes in the different blocks showed an association with AD.

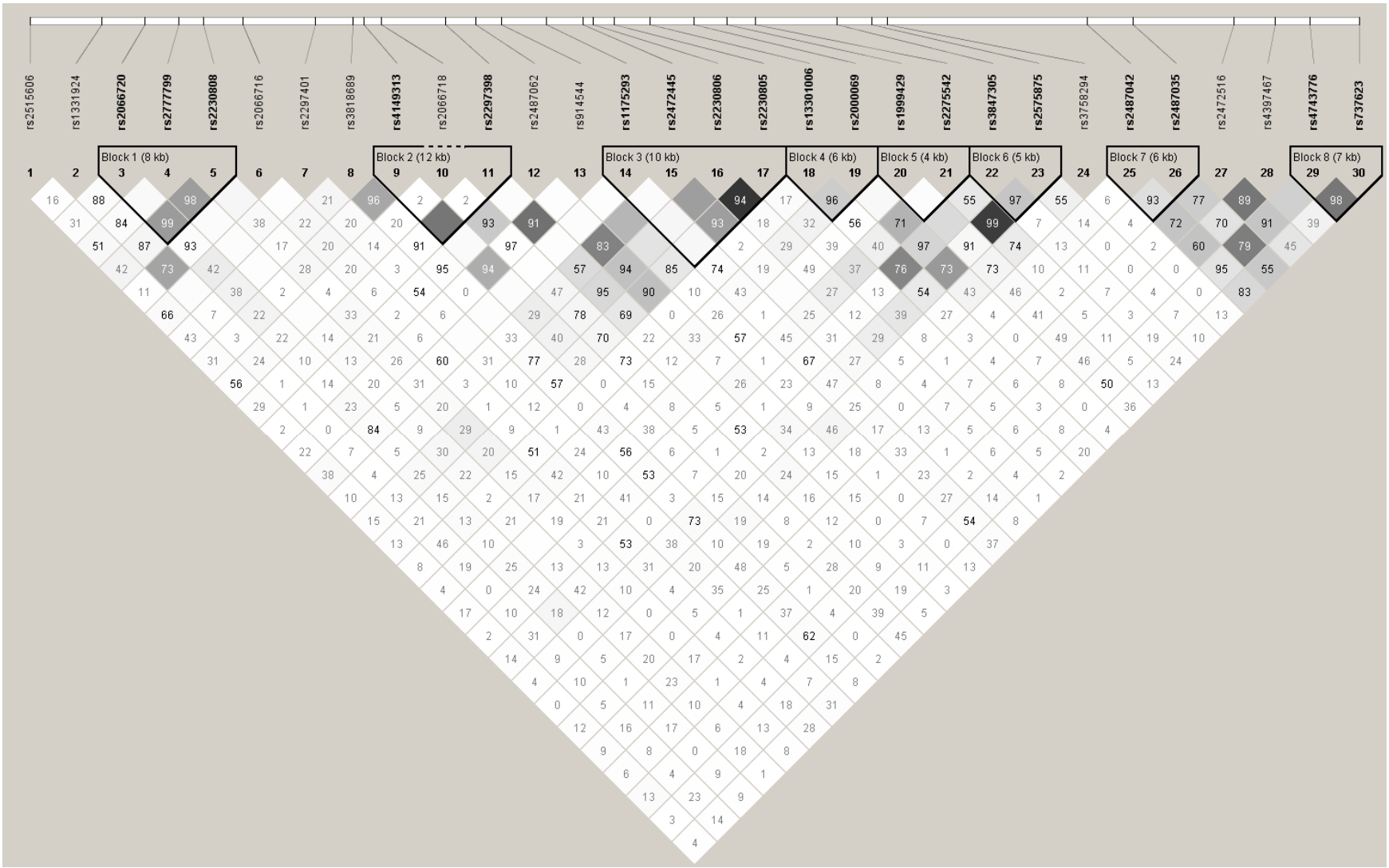

Fig. 27: LD structure of the *ABCA1* gene, including the haplotype block structure.
Values are used to represent D' whereas colors were used to encode r^2 (D' values of 1.0 are never shown - the box is empty; black = high r^2 value, white = low r^2 value). The blocks followed the haplotype block definition by Gabriel et al., (Gabriel, Schaffner et al. 2002) as implemented within Haploview 4.2.

Tab. 36: Haplotype blocks and frequencies for *ABCA1* in the Munich collective

Haplotype ID	Sequence	Frequency (Case/Control)	P-values
Block 1			
1.1	GTG	0.730, 0.739	0.683
1.2	ATA	0.127, 0.134	0.687
1.3	GCA	0.133, 0.110	0.202
Block 2			
2.1	AA	0.872, 0.893	0.231
2.2	GC	0.071, 0.061	0.466
2.3	GA	0.056, 0.045	0.354
Block 3			
3.1	AGGG	0.611, 0.614	0.909
3.2	ATAA	0.140, 0.134	0.769
3.3	AGAA	0.137, 0.124	0.482
3.4	GGGG	0.070, 0.075	0.725
Block 4			
4.1	GG	0.533, 0.556	0.401
4.2	GA	0.304, 0.313	0.715
4.3	AA	0.158, 0.130	0.141
Block 5			
5.1	CG	0.662, 0.638	0.360
5.2	CA	0.322, 0.333	0.646
Block 6			
6.1	CC	0.614, 0.614	0.997
6.2	CT	0.256, 0.259	0.904
6.3	GT	0.128, 0.125	0.844
Block 7			
7.1	GT	0.435, 0.457	0.414
7.2	GC	0.336, 0.340	0.886
7.3	AT	0.224, 0.198	0.229
Block 8			
8.1	AG	0.573, 0.562	0.666
8.2	GA	0.290, 0.290	0.991
8.3	AA	0.134, 0.146	0.506

The final analysis performed in the Munich cohort was a 2- and 3-marker sliding window to identify SNPs that contributed association signals (Fig. 28).

Two haplotypes, both including SNP # 1 which had shown association before, yielded significant p-values. The strongest association was observed in the 3-marker haplotype 1.2, also including the other formerly significant SNP # 3, generated by SNPs # 1-3 ($p = 0.025$, frequency 33.2%). Additionally, the 2-marker haplotype 1.2 generated by SNPs # 1-3 ($p = 0.027$, frequency 36.5%). For both haplotypes the 2- or 3- marker haplotype associations were clearly higher than those of the single markers. Another significant 3- marker haplotype was

observed spanning SNPs # 16-18 (haplotype 16.3) with a weak p-value of 0.047 and a low frequency of 8.2% only. None of the involved markers had shown single marker associations.

Detailed results from the 2- and 3-marker sliding window approach are depicted in the Appendix. (§ 7.4.4). Haplotypes with frequencies below 5% were not taken into account, due to their unlikely occurrence.

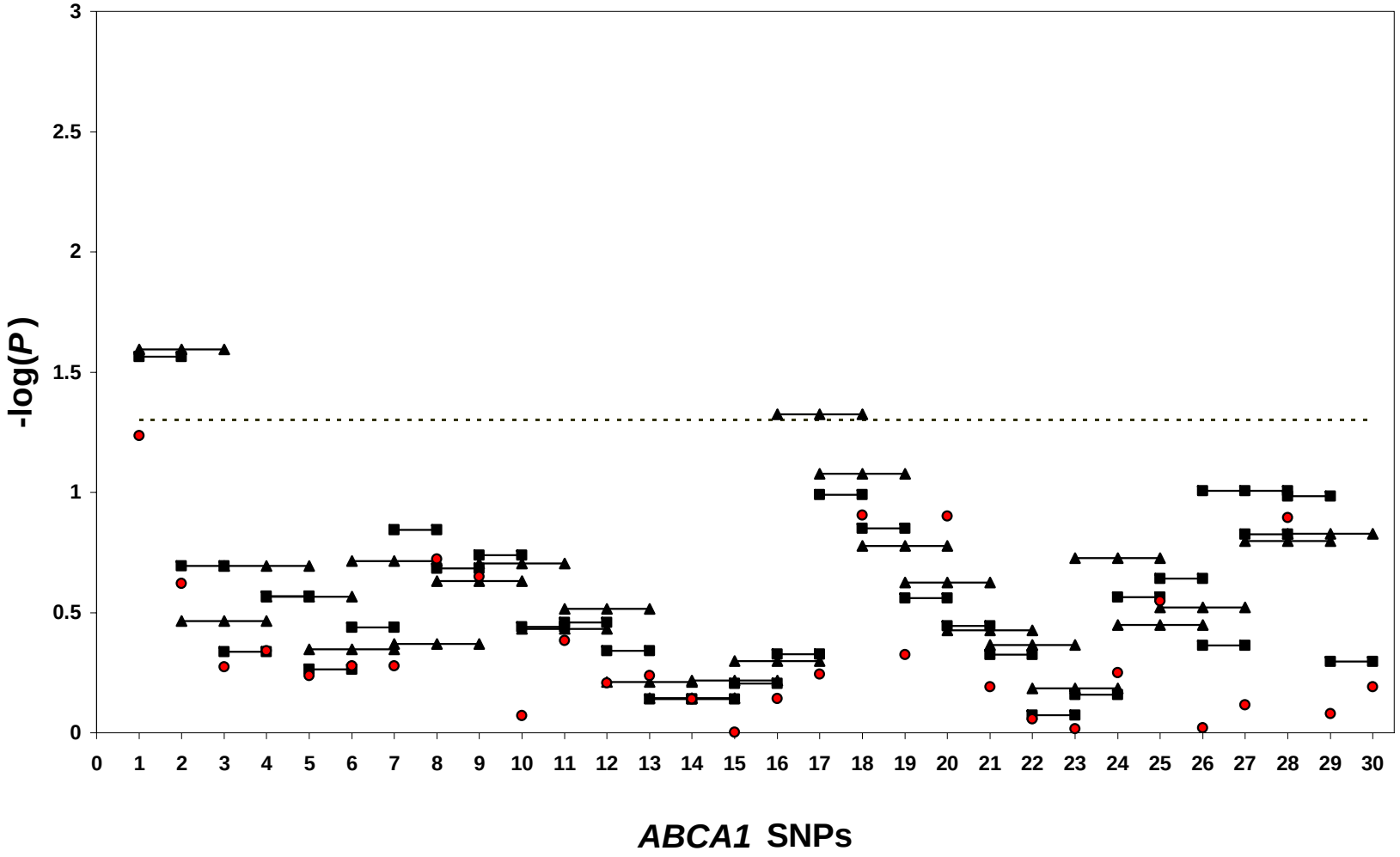

Fig. 28: Sliding window analysis of 2- and 3-marker haplotypes for *ABCA1*

Red circles represent -log p values for single marker SNPs genotyped. Lines between solid squares represent the -log p value of 2-marker haplotypes. Lines between solid triangles mark -log p values for 3-marker haplotypes. All 2- and 3-marker haplotypes as calculated with Haploview. The dotted lines at a -log p value of approximately 1.3 demarcate the threshold of p = 0.05. Numbers correspond to those assigned to SNPs by Haploview.

5.3.1.2 Replication of Association Results in Independent Studies

Based on the analysis of the Munich cohort two SNPs (# 1 and 3) were chosen for replication in independent studies. In the scope of this study a large Swedish case-control cohort (SWE, N = 883) and a German discordant sib-pairs cohort (N = 735) were available. A subsequent pooling analysis of the Munich and Swedish cohort (pooled = MUC + SWE, N = 1611) was performed to increase statistical power.

In the Swedish cohort association signals were replicated for both SNPs with clearly stronger associations than found in the Munich cohort (# 1, rs2515606: $p < 0.001$ and # 3, rs2066720: $p = 0.002$). SNP # 1 was even found to have a strong association signal ($p < 0.001$) in the pooled analysis, whereas SNP # 3 could not be replicated. Stratification of the Swedish as well of the pooled sample confirmed association of SNP # 1, in both (the EOAD and the LOAD) sub-collectives, except with weaker signals. SNP # 3 had a slightly weaker association signal after stratification, but limited to the EOAD group of the Swedish collective. All risk alleles found before within the Munich cohort were confirmed. Detailed results of the genotypic tests are depicted in Tab. 37. Results of allelic test are shown in the Appendix (§ 7.3.4, Tab. 55).

Analysis of SNPs in the sib-pairs cohort failed to replicate signals for both SNPs (# 1 and # 2). Results of the tests are depicted in Tab. 37.

Tab. 37: Results of replication for selected SNPs of *ABCA1* in independent studies – part 1

#	SWE (N=883)			Pooled Population (MUC+SWE) (N=1611)		
	AD affection genotypic p-values for major allele unless otherwise noted (associated allele); OR (95%CI) with covariates (sex and onset)			AD affection genotypic p-values for major allele unless otherwise noted (associated allele); OR (95%CI) with covariates (sex, onset, population)		
	SWE complete (N=883)	EOAD subcollective (N=220)	LOAD subcollective (N=661)	pooled complete (N=1611)	EOAD subcollective (N=501)	LOAD subcollective (N=1105)
1	**<0.001 (C); 2.08 (1.4-3.0)**	**0.021 (C); 2.33 (1.1-4.8)**	**0.005 (C); 1.93 (1.2-3.1)**	**<0.001 (C); 1.83 (1.4-2.4)**	**0.004 (C); 2.08 (1.3-3.4)**	**0.005 (C); 1.66 (1.2-2.4)**
3	**0.002 (G); 2.03 (1.3-3.2)**	**0.003 (G); 3.42 (1.5-7.8)**	0.084	0.055	0.090	0.168

Tab. 37: Results of replication for selected SNPs of *ABCA1* in independent studies – part 2

#	SNP	p values	
		fbat100	fbat010
1	rs2515606	0.927	0.713
3	rs2066720	0.115	0.271

5.3.1.3 Statistical Analyses of Clinical Measures for *ABCA1*

For the complete set of 30 SNPs linear regression analysis of clinical measures was performed, testing association between genotypes of the SNPs and age at onset and the scores on the Mini-Mental State Examination (MMSE) within the whole Munich collective. Note that MMSE scores were only available for 416 of the subjects. Age at onset and gender were included as covariates during analysis to rule out results only affected by a specific sub-population. None of the SNPs presented with significant associations. Detailed results are reported in the Appendix (§ 7.3.4, Tab. 56 and Tab. 57).

5.3.1.4 Functional Analyses for *ABCA1* in Brain Samples

In order to determine possible functional effects of the affected SNPs on ABCA1 expression, the same preliminary functional approach as used for APP before was performed in 20 brain samples (12 cases, 8 controls).

As for the replication studies, based on the analysis of the Munich cohort, the two SNPs that had shown association before (SNPs # 1 and 2), were chosen for analysis of possible functional effects.

The formerly identified associations of genotypes in *ABCA1* with AD were also tested in the small study cohort of 20 brain samples. One of the SNPs (# 3, rs2066720) confirmed the formerly found association in plasma samples in the analyzed brain samples when tested recessive for the major allele (t-test, p = 0.002). ANOVA analysis yielded no significant results for the two tested SNPs. Results are presented in the table below (Tab. 38). Due to the small sample size (n = 20) and the explorative character of the functional study, the following investigations were undertaken anyways, but results have to interpreted with caution.

Tab. 38: Results of statistical analysis of *ABCA1* genotypes in brain samples

#	SNP	p-values from ANOVA (association between genotype and phenotype)	p-values from t-test (recessive for major allele)
1	rs2515606	0.520	0.731
3	**rs2066720**	0.139	**0.002**

5.3.1.4.1 Gene Expression Analyses for *ABCA1* in Brain Samples

Using control RNA of defined concentration (Applied Biosystems FirstChoice® Human Brain Reference RNA, 1 mg/mL, Part No. AM6050), a calibration line was determined from which the RNA content of the samples was calculated (Fig. 29).

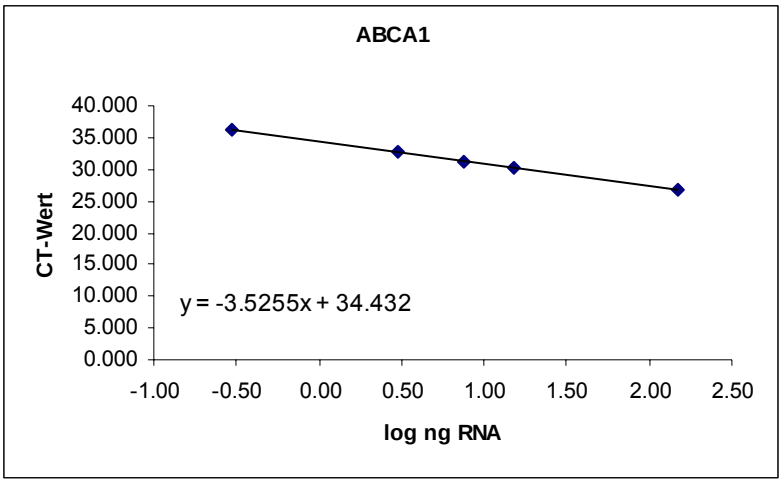

Fig. 29: Calibration line for determination of *ABCA1* mRNA levels.

Gene expression analysis in AD and control brains revealed SNP # 3 to have a significant impact on ABCA1 mRNA levels, where the G allele was associated with a higher expression (All samples: GG (n=15) 9.44±10.8 Arbitrary Units (AU), GA/AA (n=3) 0.50±0.34 AU; p = 0.006). SNP # 1 yielded no associations. The individual results for ANOVA analysis and t-test for recessive major of both SNPs are presented in the table below (Tab. 39).

Tab. 39 Results of statistical analysis of gene expression of *ABCA1*

#	SNP	p-value from t-test - association between genotype and APP mRNA level (recessive for major allel)
1	rs2515606	0.316
3	**rs2066720**	**0.006**

5.3.2 Analysis of SNPs from *LIPC*

The *LIPC* gene is located in chromosomal region 15q21-q23 and contains 9 exons spanning 158 kilobases. Since hepatic triglyceride lipase (LIPC), much like the major genetic risk factor for Alzheimer's disease (AD), apolipoprotein E (ApoE), is associated with altered lipid metabolism, it is a potential functional candidate for AD risk.

The potential association of the *LIPC* gene in AD was thoroughly investigated by utilizing a fine mapping approach across the entire *LIPC* gene in a large German case-control series.

After several thorough databank researches, 30 SNPs were selected (Tab. 40). From these SNPs, 25 could be confirmed to be polymorph in the Munich collective, achieved a sufficient genotype frequency (>80%) and showed no deviation from the HWE in controls (Tab. 41 and Fig. 30).

Tab. 40: Selected SNPs from the *LIPC* gene.

SNPs that yielded insufficient genotype frequencies (<80 %), significant deviations from HWE and minor allele frequencies <0.001 were not taken into account for the statistical evaluations. SNPs passing all aforementioned criteria are highlighted in bold font. Alleles are reported in alphabetical order as found on http://snpper.chip.org.

SNP ID NCBI	SNP Position NCBI	Alleles	Role
rs17468168	56501641	C/T	Promoter
rs4774297	**56507723**	**A/C**	**Promoter**
rs1800588	**56510967**	**C/T**	**Promoter**
rs17190517	**56516436**	**C/T**	**Intron**
rs261341	**56518859**	**A/G**	**Intron**
rs483140	**56524427**	**C/G**	**Intron**
rs261336	**56529710**	**C/T**	**Intron**
rs3825776	**56534122**	**A/G**	**Intron**
rs10518976	**56538493**	**A/T**	**Intron**
rs10518978	**56543465**	**C/T**	**Intron**
rs6494011	**56547936**	**C/G**	**Intron**
rs1968685	**56555046**	**C/G**	**Intron**
rs6494013	56560978	C/G	Intron
rs1869137	**56565704**	**C/G**	**Intron**
rs4561401	56571369	C/T	Intron
rs1973028	**56578646**	**A/G**	**Intron**
rs1869144	56584592	A/G	Intron
rs2899631	**56589377**	**C/G**	**Intron**
rs4774302	**56595870**	**C/T**	**Intron**
rs2899632	**56599161**	**A/G**	**Intron**
rs12899928	**56606843**	**C/G**	**Intron**
rs4775072	**56613005**	**C/T**	**Intron**
rs1971544	56616743	C/G	Intron
rs6078	**56621285**	**A/G**	**Coding exon**
rs2242064	**56626564**	**G/T**	**Intron**
rs871804	**56631506**	**A/G**	**Intron**
rs2414594	**56638063**	**A/G**	**Intron**
rs3829462	**56640371**	**G/T**	**Coding exon**
rs6074	**56648255**	**A/C**	**Coding exon**
rs4774308	**56653448**	**C/T**	**3' UTR**

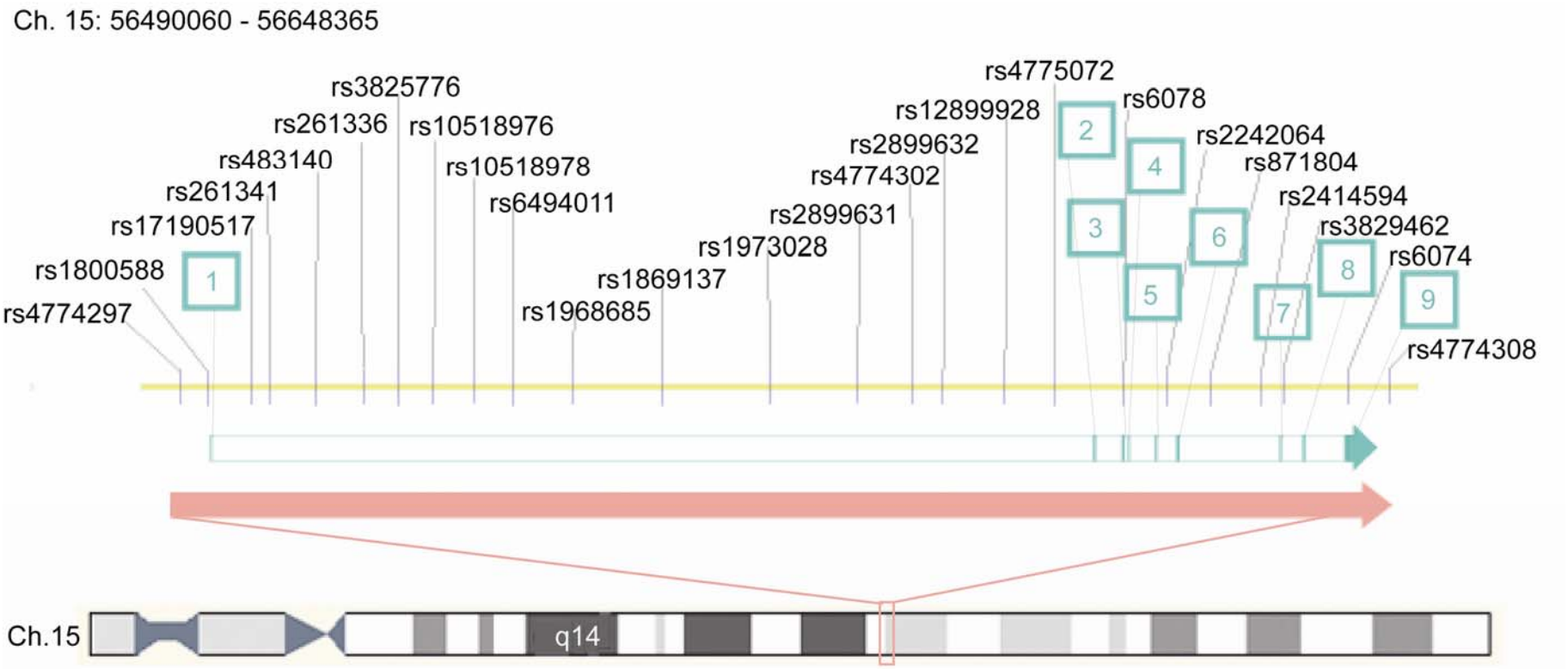

Fig. 30: Schematic of the *LIPC* gene.

The positions of the analyzed SNPs (shown with rs numbers) and exons in the *LIPC* gene are shown. Exons are marked with green bars on the forward strand from left to right. The arrow shows direction of transcription.

Single marker and haplotype associations were explored. Single marker analysis within Haploview of the 25 *LIPC* SNPs (Tab. 41) revealed no allelic associations with AD. One SNP within *LIPC* presented with a weak genotypic association with AD (rs483140; p=0.039), although this did not survive correction for multiple testing. No further significant association for genotypic single markers was found (Tab. 42). The major allele frequencies (Tab. 41), as well as genotype frequencies (Tab. 42) were comparable in cases and controls and showed no notable differences.

Also no significant association when stratifying the patient sample in the subgroups of EOAD and LOAD observed (according to the age at onset; see Appendix, § 7.3.5, Tab. 58).

Tab. 41: Results from allelic tests for *LIPC* gene.

Allelic associations were tested for significance using χ2-tests, all as implemented in Haploview version 4.2. All shown SNPs yielded genotype frequencies of at least 80 %, no significant deviation from HWE and minor allele frequencies had to be at least 0.001.

#	SNP	Associated allele	Major allele frequency MUC complete (cases, controls)	Allelic p-values
1	rs4774297	A	0.537, 0.535	0.938
2	rs1800588	T	0.773. 0.805	0.155
3	rs17190517	C	0.557. 0.542	0.597
4	rs261341	A	0.708. 0.719	0.643
5	rs483140	G	0.736. 0.692	0.068
6	rs261336	G	0.819. 0.854	0.084
7	rs3825776	T	0.677. 0.668	0.726
8	rs10518976	T	0.916. 0.911	0.773
9	rs10518978	T	0.883. 0.897	0.456
10	rs6494011	C	0.847. 0.845	0.941
11	rs1968685	C	0.579. 0.558	0.507
12	rs1869137	C	0.570. 0.586	0.563
13	rs1973028	T	0.649. 0.625	0.340
14	rs2899631	G	0.794. 0.833	0.079
15	rs4774302	C	0.625. 0.627	0.933
16	rs2899632	A	0.805. 0.822	0.400
17	rs12899928	G	0.980. 0.977	0.695
18	rs4775072	C	0.981. 0.979	0.744
19	rs6078	A	0.962. 0.969	0.498
20	rs2242064	G	0.685. 0.677	0.744
21	rs871804	T	0.876. 0.883	0.661
22	rs2414594	G	0.991. 0.983	0.169
23	rs3829462	A	0.991. 0.984	0.266
24	rs6074	A	0.874. 0.880	0.760
25	rs4774308	C	0.629. 0.644	0.555

Tab. 42: Results from genotypic tests for the *LIPC* gene.

Genotypic associations were tested for significance using a logistic regression recessive model [i.e. AA vs Aa,aa; where major allele is A] and are adjusted for age and gender (each SNP independently) all as implemented in SPSS.

SNP	Alleles (Major/Minor)	Genotype Frequency						
		Cases			Controls			p-values
		AA	Aa	aa	AA	Aa	aa	
rs4774297	A/C	0.307	0.458	0.235	0.297	0.472	0.231	0.664
rs1800588	C/T	0.604	0.339	0.058	0.662	0.285	0.053	0.130
rs17190517	C/T	0.327	0.459	0.214	0.336	0.412	0.252	0.731
rs261341	A/G	0.509	0.397	0.094	0.536	0.366	0.098	0.491
rs483140	C/G	0.532	0.408	0.06	0.458	0.468	0.074	**0.039**
rs261336	C/T	0.664	0.311	0.025	0.736	0.236	0.028	0.055
rs3825776	A/G	0.462	0.429	0.109	0.437	0.462	0.101	0.571
rs10518976	A/T	0.834	0.162	0.005	0.827	0.166	0.007	0.978
rs10518978	C/T	0.794	0.179	0.027	0.819	0.155	0.025	0.853
rs6494011	C/G	0.716	0.260	0.023	0.704	0.282	0.014	0.733
rs1968685	C/G	0.347	0.464	0.189	0.345	0.427	0.228	0.904
rs1869137	C/G	0.303	0.533	0.163	0.340	0.486	0.174	0.375
rs1973028	A/G	0.427	0.446	0.127	0.394	0.463	0.143	0.361
rs2899631	C/G	0.654	0.279	0.066	0.696	0.274	0.03	0.364
rs4774302	C/T	0.395	0.462	0.143	0.375	0.505	0.12	0.522
rs2899632	A/G	0.644	0.319	0.037	0.678	0.287	0.035	0.286
rs12899928	C/G	0.963	0.034	0.002	0.955	0.045	0.000	0.581
rs4775072	C/T	0.965	0.033	0.002	0.958	0.042	0.000	0.625
rs6078	A/G (V95M)	0.926	0.072	0.002	0.937	0.063	0.000	0.600
rs2242064	G/T	0.476	0.418	0.106	0.444	0.465	0.091	0.431
rs871804	A/G	0.77	0.207	0.023	0.784	0.199	0.017	0.794
rs2414594	A/G	0.982	0.018	0.000	0.965	0.035	0.000	0.336
rs3829462	G/T (F356L)	0.979	0.018	0.002	0.969	0.031	0.000	0.591
rs6074	A/C	0.775	0.200	0.025	0.782	0.196	0.021	0.957
rs4774308	C/T	0.398	0.464	0.138	0.419	0.455	0.126	0.544

On basis of the 25 SNPs covering the complete genetic region, the LD structure for the *LIPC* gene was determined. The average inter-marker distance was calculated to be 6.1 kb. It could be shown that the entire genomic region at LIPC, from the promoter SNP rs4774297 down to rs4774308, located downstream from LIPC, showed considerable variability in D' values, thereby indicating a large DNA region of variable recombination. Likewise, the r^2-values for the pairwise comparisons are also highly variable, hence suggesting the presence of numerous marker blocks across the entire LIPC region Within the genotyped region, a total of 6 LD blocks were identified on basis of the D' and r^2 values (Fig. 31). For each of the the blocks haplotypes were calculated as depicted in the table below (Tab. 43). The rarest estimated haplotype of block 1 showed an association with AD (p-value 0.025), but with a frequency of only 4.8% in cases and 7.6% in controls.

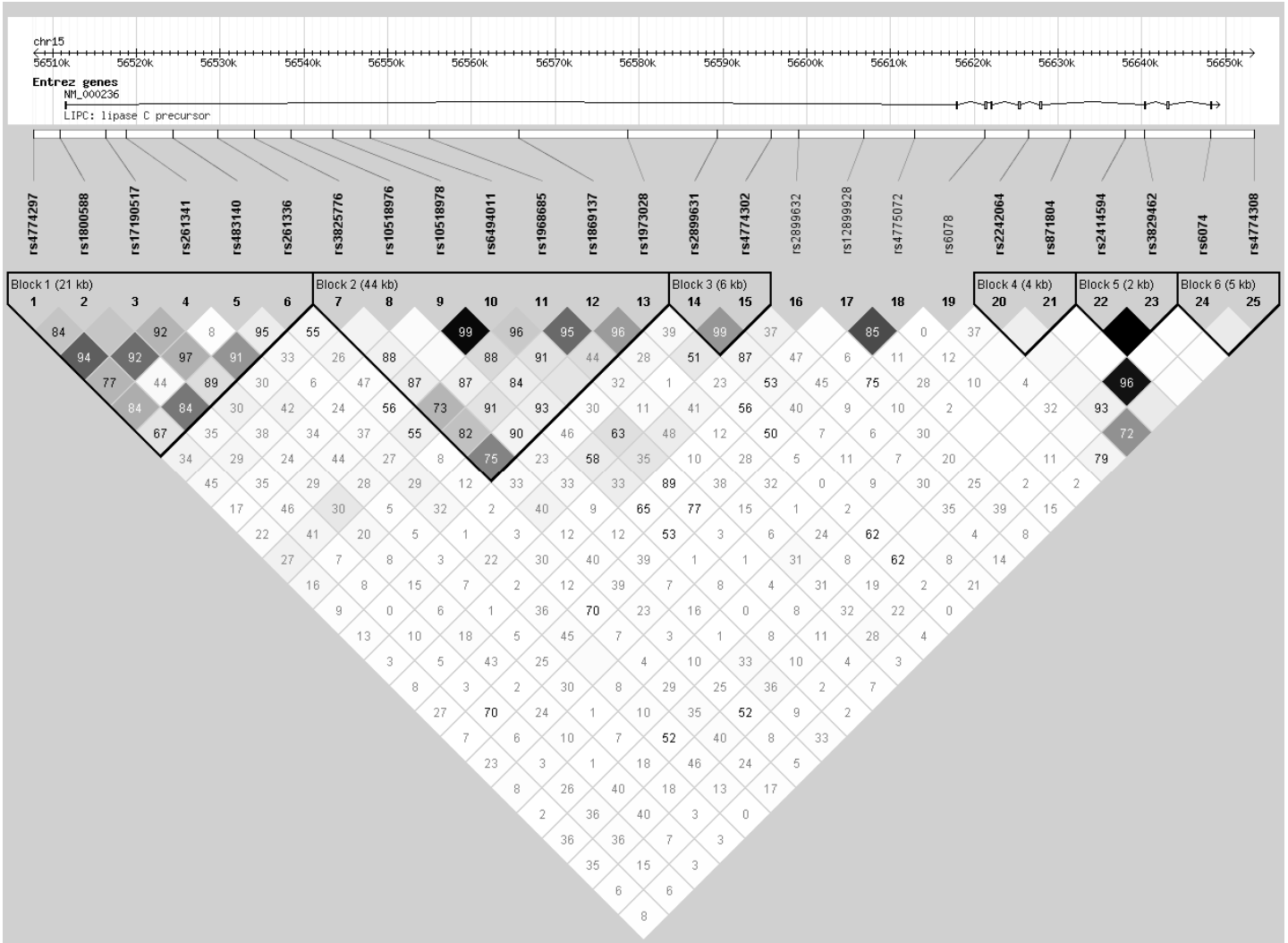

Fig. 31: LD structure of the *LIPC* gene, including the haplotype block structure.
Values are used to represent D' whereas colors were used to encode r^2 (D' values of 1.0 are never shown - the box is empty; black = high r^2 value, white = low r^2 value). The blocks followed the haplotype block definition by Gabriel et al., (Gabriel, Schaffner et al. 2002) as implemented within Haploview 4.2.

Tab. 43: Haplotype blocks and frequencies for *LIPC* in Munich collective

Haplotype ID	Sequence	Frequency *(Case/Control)*	P-values
Block 1			
1.1	ACTGGA	0.414, 0.426	0.647
1.2	CCCGCA	0.164, 0.172	0.680
1.3	CTCAGG	0.142, 0.116	0.150
1.4	**CCCACA**	**0.048, 0.076**	**0.025**
Block 2			
2.1	TTCCCCT	0.307, 0.299	0.741
2.2	CTCCGGC	0.242, 0.255	0.568
2.3	TTTGGGT	0.139, 0.137	0.918
2.4	TACCCCT	0.079, 0.081	0.913
2.5	TTCCCGC	0.067, 0.068	0.960
Block 3			
3.1	CT	0.626, 0.629	0.898
3.2	GC	0.207, 0.176	0.142
3.3	CC	0.167, 0.195	0.174
Block 4			
4.1	GC	0.560, 0.560	0.999
4.2	TC	0.316, 0.324	0.750
4.3	GT	0.125, 0.117	0.651
Block 5			
5.1	GA	0.991, 0.983	0.169
Block 6			
6.1	CT	0.503, 0.524	0.439
6.2	CC	0.371, 0.356	0.562
6.3	AT	0.126, 0.120	0.742

Results of the 2- and 3-marker sliding window approach to identify SNPs that contributed association signals are depicted in the Figure below (Fig. 32). Detailed results from the 2- and 3-marker sliding window approach are depicted in the Appendix. (§ 7.4.5). Haplotypes with frequencies below 5% were not taken into account, due to their unlikely occurrence. One significant marker peak was observed spanning SNPs # 14-17. The strongest association was observed in the 3-marker haplotype 14.4 generated by SNPs # 14-16 ($p = 0.0065$, frequency 7.1%). None of the involved markers had shown single marker associations. The haplotypes including SNP # 5, yielding a significant genotyping p-value before, yielded no significant results.

Since the significant observations did not emerge for the same SNPs in different approaches, no replication in independent studies was performed.

Since occurrence of this very rare haplotype is too unlikely, the result can not be regarded as a confirmation of the former association of SNP # 5. Since no other approach (sliding window, stratification, allelic association) confirmed the finding (also sliding window was the only observation of significance in the region spanning SNPs # 15-17), replication in another sample was not carried out. Moreover the p-value is only borderline significant (only slightly below 0.05). Additionally, literature does not suggest positive findings and LIPC is not directly involved in AD pathogenesis.

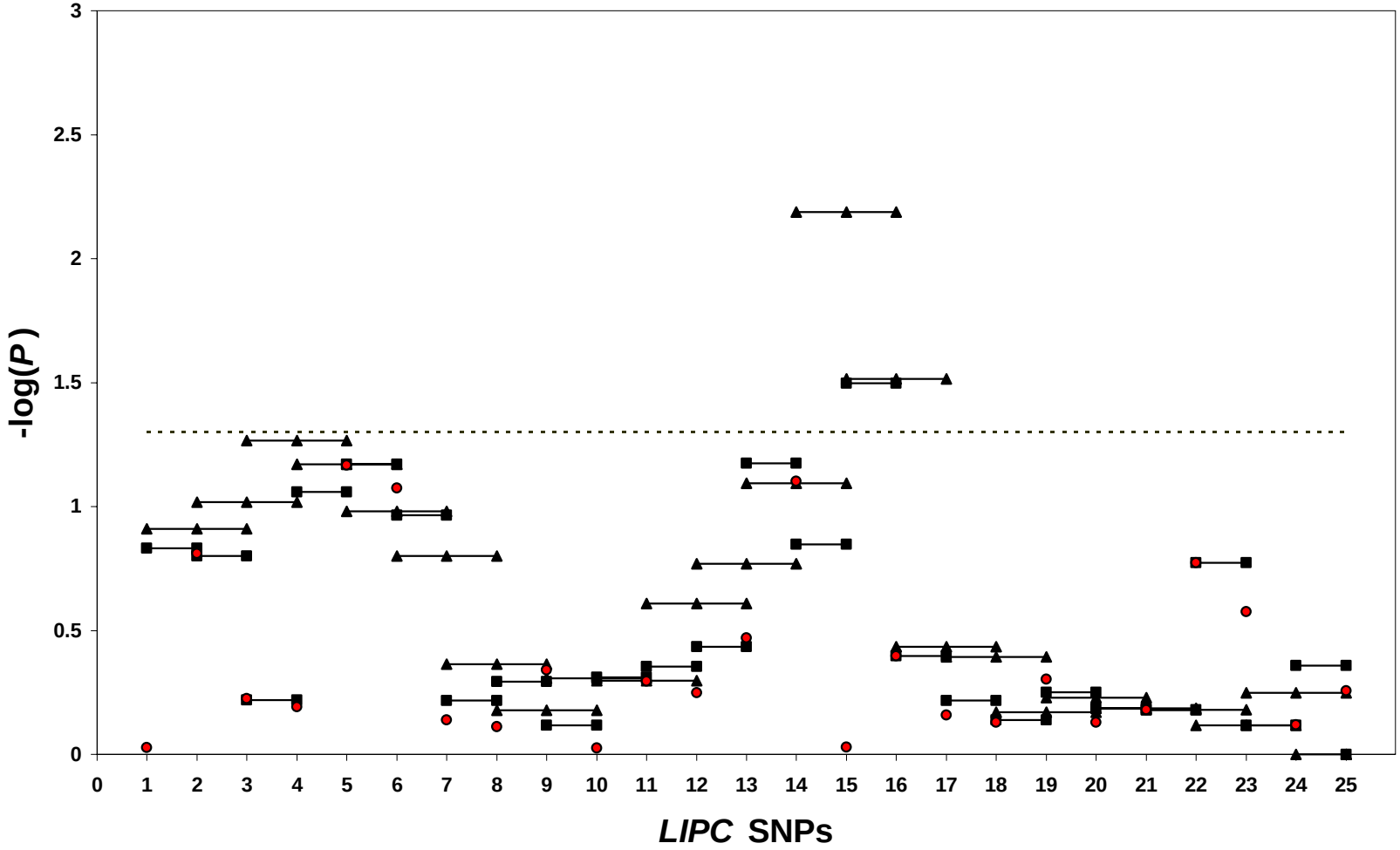

Fig. 32: Sliding window analysis of 2- and 3-marker haplotypes for *LIPC*

Red circles represent -log p values for single marker SNPs genotyped. Lines between solid squares represent the -log p value of 2-marker haplotypes. Lines between solid triangles mark -log p values for 3-marker haplotypes. All 2- and 3-marker haplotypes as calculated with Haploview. The dotted lines at a -log p value of approximately 1.3 demarcate the threshold of p = 0.05. Numbers correspond to those assigned to SNPs by Haploview.

6 Discussion

Complex diseases like Morbus Alzheimer can not be traced back causally to one mechanism, but result from the interaction of different genetic, environmental and lifestyle dependant risk factors (Brown, Lockwood et al. 2005). Mutations in the genes *PS1*, *PS2* and *APP* cause an increase in Aβ42 production, and therefore increase the deposit of amyloid plaques, one of the most important characteristics of AD (Ozturk, Minster et al. 2007). Another indication for an association with AD is constituted by numerous studies, proofing the ε4 allele in the *ApoE* gene to be the strongest risk factor for LOAD and sporadic forms of AD, respectively (Rebeck, LaDu et al. 2006).

It can be assumed, that in addition to the above mentioned genetic risk factors many other genes or their mutations contribute to the development and course of Alzheimer´s disease. Preferentially, genes were analyzed, whose mutation could, according to nowadays knowledge, trigger the neuro-pathological process typical for AD or could influence the course of disease. Since former studies have proven a correlation between occurrence of AD and presence of Aβ (Hardy and Higgins 1992), focus of the presented work was the *APP* gene and genes involved in the processing of APP and Aβ formation. Additionally, genes involved in the lipid metabolism functionally similar to the major genetic risk factor for Alzheimer's disease, apolipoprotein E (ApoE), were taken into consideration during selection of candidate genes. Moreover, results from genome-wide linkage studies (Myers, Holmans et al. 2000; Lee, Cheng et al. 2006) were taken into account for selection of candidate genes, tested for their association with AD and subsequently functionally characterized.

Subsequently, quality of the data, study design, gained results of association and functional analyses, as well as relevance of the gained data for the development of Morbus Alzheimer are discussed.

6.1 Advantages and Disadvantages of the Selected Study Designs

Disease relevant genes can be identified by means of association studies. For this purpose two approaches can be used: the case-control and the family based study design. The most common study type in epidemiology is the case-control study with independent and unrelated samples (Lewis 2002). In the present work a case-control association study within a

German population was carried out, whose results served as starting point for further analyses. For exclusion of possible erroneous association results, which can occur in a case-control study, significant results were validated in another case-control study, as well as in a sib study. Both approaches have advantages and disadvantages.

Genetic markers (SNPs) are usually identified by means of a case-control-study, resulting in significant differences regarding allele and genotype frequencies between affected individuals (cases) and independent healthy controls (Lewis 2002). For obtaining the same information in a sib pair approach, healthy siblings of affected individuals are included as control group (Whittemore and Tu 2000). For the investigation of disease with a long latency, as well as for clarification of reasons for an acute disease outbreak, case-control-studies are an important resource. In comparison to sib pair studies, case-control-studies are not as laborious and therefore cost-saving. Hence, examination of a large sample size within relatively short time is possible. However, careful selection of adequate controls is of utmost importance. Cases and controls have to be matched dependent on some major characteristics (same population group, age, gender, socio-economic status, etc.), so that both groups are comparable (Lewis 2002). Thereby, structural differences in the matching variables are eliminated, although stratification problems can not be completely precluded. Stratification is the observation, when cases and controls show a diversity in allele frequencies for markers, due to population structures which can not be ascribed to the disease. This can often lead to erroneous association results, which later may not be confirmed by independent experiments (Schork, Fallin et al. 2001). Population effects were minimized by stratification using appropriate tests. A regression analysis was chosen, since population characteristics, such as gender and age at onset, can be incorporated as covariates during examination. Additionally, deviations from the Hardy-Weinberg-equilibrium (HWE) within the control group and differing genotype frequencies between study cohorts can be a hint for stratification effects and was therefore calculated (Bickeboller and Fischer 2007).

To rely entirely on data gained from case-control-studies has also disadvantages. Only one outcome can be examined at a time (Lewis 2002). Moreover, no prevalence or incidence estimation can be ascertained from a case-control approach, since the case / control ratio is defined by the amount of subjects included in the study and not by the frequency of cases in the population. Major sources for appointment of subjects to “cases” or “controls” are patient´s records and interviews with patients and their relatives. A differing ability of memory of cases and controls can cause a falsification, which is called “recall bias” (Brown, Lockwood et al. 2005). Another form of information bias can occur in those studies, where

not the affected persons are questioned concerning anamnesis of disease, but their family members and persons in support. Often, no detailed information about exposition, environmental and life style conditions are available in those cases (substitute-interview bias).

An important advantage of the sib pair approach is the common genetic background of the participants. Statistical analysis of genotypes is conducted via „Sib Transmissions-Disequilibrium Test“ (S-TDT) (Spielman and Ewens 1998), a modified version of the „Transmissions-Disequilibrium Tests“ (TDT) (Spielman, McGinnis et al. 1993) (see § 2.1). For this purpose, genotypes of the affected persons are compared directly to those of their healthy siblings. Therefore, falsifications, developed by stratified populations, can be avoided. Unlike case-control studies, accomplishment of this study type is costly in terms of time, since at least two family members – one affected and at least one healthy brother or sister – have to be recruited. In practice, extensive documentation of kinsmanlike relations of study participators forms another problem. Since only those siblings with demonstrated differences in allele frequencies can be incorporated in the statistical association analysis, a very low statistical power is the result (Whittemore and Tu 2000). In contrast, information about allele frequencies from all the subjects can be used in case-control studies.

As a basis for the presented work, a case-control study design was utilized. Therefore, incorporation of a necessary great number of subjects into the study was performed, resulting in a sufficiently high power. Recruiting of informative sib pairs was hindered, because of the late age at onset for AD. Nevertheless, for exclusion of optionally occurring stratification problems, significant association results from the Munich sample were tested in two additional Caucasian samples – in an independent case-control cohort from Sweden and in one sib pair cohort from Germany.

6.2 Is the Selected Study Approach still Up-to-date?

The chosen approach, described and discussed in the paragraph above, was the high-level choice when the practical part of this study was started in 2005. Meanwhile, analyzing methods, technologies and especially throughput have been improved. Nowadays, a different approach would have been used: a genome-wide association study (GWAS). In the formerly applied method typically few or at most some hundred markers located in each others vicinity could be analyzed at once. In contrast, a GWAS enables analysis of up to 10^5 – 10^6 SNPs

distributed over the whole genome at the same time, hence representing a superb approach for multi-factorial diseases involving several genes, such as Alzheimer´s Disease (AD). Another advantage of GWAS is, that it can also be carried out, when no candidate regions from linkage studies are known (Bickeboller and Fischer 2007).

6.3 Summary and Discussion of the Main Results

Genotyping data for the individual genes were analyzed by means of relevant statistical tests as described before (see § 2.16) and significant association results were replicated in independent studies. Although it is commonly used for association studies, multiple testing of the significant results according to the Bonferroni method was abandoned. This is due to the fact that the Bonferroni method decreases the α-significance level, so that the cumulative (and not the individual) significance threshold is 0.05. In this approach the significance threshold would have been divided by the total number of tested SNPs for each gene, e.g. decreasing the threshold for APP to 0.001 (= 0.05 / 39 SNPs). The disadvantage of such α adjustments is the decline of power of the tests. The probability to discover actually existent differences by multiple testing decreases with the amount of hypotheses to be tested. Thus, only highly significant results can lead to rejection of the null hypothesis.

Since AD is a multifactorial disease and the genes that were investigated in the present study are none of the main genes, statistical results are unlikely to be highly significant, because the disease is probably not only triggered by one SNP variant alone, but by a combination of different variants. Moreover, due to the relatively high throughput, the amount of tested SNPs is very high and the significance level would hence be accordingly low. Therefore, multiple testing is not applicable for this approach. The crucial principle for the verification of a true association between polymorphisms and phenotype for complex diseases as AD, is the replication of the statistical significant results in independent cohorts.

In the present work several SNPs yielded statistical significant associations (p-value < 0.05), which would have not survived multiple testing. Nevertheless, validity of the significant findings from the screening sample could be confirmed by means of successful replication in independent study cohorts, which is of utmost importance and the better approach.

In the following paragraph the results are also compared to those from other groups. Discrepancies between the results from different studies are mainly suggestive of the heterogeneity of complex genetic diseases. Different study populations can differ in sample size, study designs, mean age at onset of subjects, gender distribution within the cohort, allele frequencies and correlation structures. In addition, study populations are exposed to different environmental conditions. All that are reasons for unequal results and can be quantitatively brought to one result by a meta-analysis (Crowther and Cook 2007).

6.3.1 Summary and Discussion of Results for *APP*

The *APP* gene represents a strong positional and functional candidate for AD risk on many levels. Linkage studies have provided strong evidence for a susceptibility loci potentially predisposing to LOAD in the vicinity of the *APP* gene on chromosome 21 (Myers, Wavrant De-Vrieze et al. 2002; Olson, Goddard et al. 2002; Blacker, Bertram et al. 2003). APP is the parent protein from which Aβ is derived and coding mutations in *APP* or duplications of the gene have been associated with EOAD (Goate, Chartier-Harlin et al. 1991). Furthermore, mutations in other familial AD genes, such as PSEN1, lead to altered APP metabolism (Pastor and Goate 2004) and *APP* locus duplication in trisomy 21 can result in elevated levels of circulating Aβ peptide (Schupf, Patel et al. 2001). Additionally, complete or partial trisomy of chromosome 21 leads to Down Syndrome including AD pathology, however only when the *APP* gene is present in three copies (Rumble, Retallack et al. 1989; Prasher, Farrer et al. 1998). All this evidence suggests that factors such as genetic polymorphisms, that impact upon APP processing, may exacerbate the risk for AD. In this study we have attempted to comprehensively investigate the genetic role of *APP* in AD by genotyping 39 SNPs covering the complete genetic region of the *APP* gene in a large case-control cohort. The initial single-marker, haplotype and sliding-window analyses revealed association signals within two marker peak regions (SNP # 14 – 25 and # 33 – 37) spanning from intron 3 to 8 and intron 1, respectively. A selection of SNPs underwent replication in a second large independent case-control series, including a pooled analysis, as well as an independent sib-pairs cohort. The replication analyses confirmed signals in SNPs # 16, 17, 20, 21, 23, 25, 35 and 36 and therefore their implication to Alzheimer´s disease.

One SNP (SNP # 9, rs1783016) outside the aforementioned marker peak regions, located in intron 14, yielded a slight association in the early onset AD sub-collective of the

Munich cohort, which was confirmed when tested for association with clinical measures. Identification of genetic factors, which induce an early onset of disease are of special interest. The associated allele of SNP # 9 was coupled with an approx. 2 year later age of onset; however it was also associated with a lower score on the MMSE, suggesting a more rapid decline in cognition in carriers of the rs1783016-G allele.

SNPs from the peak regions underwent further analyses in brain samples to ascertain the functional implications of polymorphisms across the APP gene on the transcriptional level (gene expression), the translational level (Western Blot) and the post-translational level (ELISA for Aβ isoforms). Effects on *APP* expression was apparent in one SNP, (# 25, rs1041420) located in intron 3. In the case of SNP # 25 the risk allele (T) was associated with elevated *APP* mRNA levels in the brain; however no significant difference was observed with respects to altered APP protein levels or soluble Aβ levels in the same samples. These findings suggest that the rs1041420-T risk allele might contribute to the development of AD due to altered amounts of APP substrate and not due to altered APP processing, as seen in *APP* gene duplication (Schupf, Patel et al. 2001) and trisomy21 (Rumble, Retallack et al. 1989; Prasher, Farrer et al. 1998).

Although none of the samples tested on the Western blot showed significant differences, an association can not be ruled out. The applied assay may not have enough sensitivity to detect existing density distinctions. Nevertheless, the Western blot approach confirmed the clear elevated presence of Aβ in brain samples from affected subjects in comparison to healthy subjects, where no Aβ was present (Hardy and Selkoe 2002).

Another observation during functional analysis was made for SNPs # 35 and # 36 (rs6516727 and rs2830099), where total Aβ levels were lower in carriers of the risk alleles in the brain samples, but no significant associations were observed for *APP* mRNA levels. The observed Aβ levels seem to be counterintuitive, since one would expect higher Aβ levels in carriers of the risk allele. This observation may be explained by the fact that only soluble (unbound) Aβ can be measured by the applied ELISA and the majority of present Aβ42 may already have formed plaques in the affected brains. Moreover, an association in the same direction as for total Aβ was made for Aβ40. This also suggests, that the protective allele favors the formation of the soluble Aβ40, which does not form neuritic plaques (Verdile, Fuller et al. 2004). Therefore one might nevertheless argue, that the risk alleles (rs6516727-T and rs2830099-G) are rather associated with altered processing than higher substrate levels, since no affect on the gene expression was observed. Moreover, the small number of brain

samples did not mirror the significant association of the genotypes and the phenotypes found in the Munich and Swedish case-control cohorts, another plausible explanation why the results seem not to fit perfectly. In another approach, SNP # 36 revealed a significant association of homozygosity of the major allele (G) with elevated CSF Aβ42 levels in a set of 85 cerebrospinal fluid (CSF) samples from AD patients, fitting to the lower Aβ levels that were found in carriers of this allele in brain samples (Bouwman, Schoonenboom et al. 2008).

Overall, the applied functional approach was well-chosen and is applicable for our experiments, since it can investigate the effect of the SNP variants on different stages (transcriptional, translational and processing level). Therefore, the implication of the present allele on altered APP expression or altered processing can be discovered. The fact, that the ELISA applied in brain samples can only detect soluble forms of Aβ combined with the small sample sizes, suggest that further study is required for confirmation of the results, in particularly the measurement of both soluble and insoluble fractions of Aβ42 in larger sample sizes. Moreover, all performed experiments in brain samples need to be repeated in a greater collective, since a too small sample size harbors the risk of introducing a bias due to random allele occurence or technical problems, which may not be representative of the real population structure.

From the data analysis performed in the scope of this study, it is assumed, that common variations within the *APP* gene contribute to the development of AD, due to increased *APP* expression or plaque formation.

There has previously been only one major study of polymorphisms across the entire *APP* gene (Nowotny, Simcock et al. 2007). Unlike the current study this prior study found no evidence for association with LOAD for either single SNPs or haplotypes in a non-stratified case-control cohort and only a weak evidence of association in an ApoE ε4 positive subset. The current study overlapped the prior study in seven instances and in all but one case the same association signal was observed in the non-stratified cohort. In six of these SNPs (SNP # 14, 19, 21, 28 and 29 in the present study) no association was observed in both studies. However, in two cases, SNP # 17 (rs2830012) and SNP # 26 (rs2830046), association results differed in the two studies. For SNP # 26 (rs2830046), a weak association signal was observed for the study of Nowotny, when stratified by ApoE genotype (present in ε4 carriers only), whereas no such observation was made in our Munich collective. The other exception was observed for SNP # 17 (rs2830012), which was present with a weak association signal in the pooled analysis of this study and was not associated in the prior study of Nowotny and co-workers. Correlation structures were comparable in both studies with LD blocks defined in

the same regions. A conservation of LD patterns across different European samples had been observed by Mueller and co-workers (Mueller, Lohmussaar et al. 2005). The collective used by Nowotny was no European sample, but a Caucasian sample from the Washington University Hospital. Therefore, our observation suggests a conservative LD structure in the region of the *APP* gene on chromosome 21q21 not only throughout different European samples, but also throughout Caucasian samples in general.

The lack of association signals observed in the prior study was suggested to be possibly a result of selection criteria excluding individuals most likely to carry a LOAD risk factor in the *APP* gene, in particularly a recent history of a stroke. However, this exclusion criterion was also applied during the recruitment process of this study. A second caveat of the prior study was that it was designed to test whether common variations in *APP* explain any risk for AD and thus it could not rule out the possibility that multiple rare variants in *APP* may be responsible, as suggested by another study (Theuns, Brouwers et al. 2006). Likewise, this current study cannot rule out additional rare variants being associated with AD; however, the association signals that were observed, were located in SNPs with a minor allele frequency between 20 to 30%.

In summary, this study is one of the two largest and most comprehensive analyses of the *APP* gene, as a risk factor for sporadic AD, reported to date. In contrast to the other study (Nowotny, Simcock et al. 2007) we observed evidence for an association with sporadic AD, which was replicated in additional sample sets. Additionally, we observed evidence to suggest that these SNPs have an impact on cognitive measures and both *APP* expression and processing. The applied functional approach seems to be adequate for the research of functional implications, although replication in a larger cohort of brain samples is of utmost importance to thoroughly investigate involvement of the analyzed SNPs. A sample size of 20 does not give the statistical power to draw final conclusions, but only can give hints or show tendencies. Unfortunately, since brain samples from a sufficient number of AD patients and healthy controls are very difficult to obtain, this approach could not be undertaken during this study.

6.3.2 Summary and Discussion of Results for Genes Involved in APP Processing – *BACE1* and *ADAM10*

Both *BACE1* and *ADAM10* represent, due to their involvement in APP processing and location in the vicinity of suggestive linkage regions, strong functional and positional candidate genes for AD (Luo, Bolon et al. 2001; Blacker, Bertram et al. 2003; Scott, Hauser et al. 2003; Postina 2008). Using a carefully ascertained case-control sample we performed a whole gene approach by genotyping 38 SNPs (*BACE1*, n =11; *ADAM10*, n = 27) that covered the entire genomic region of both genes. Single marker as well as haplotype or sliding-window analysis did not reveal evidence of a significant association of common *BACE1* or *ADAM10* variants with sporadic forms of AD. This current study represents the most thorough analysis undertaken of the *ADAM10* gene. The only prior study to our knowledge presented data on only 4 polymorphisms, but likewise no significant association with AD was observed (Prinzen, Muller et al. 2005). Prior studies of the *BACE1* gene have focused primarily on exon 5 polymorphisms, the most widely studied of which, rs638504, has shown inconclusive results (Bertram, McQueen et al. 2007), however, in this study we found no association regardless of ApoE genotype. Only one prior study, which had an approximate 50% overlap of SNPs with the current study, used a similar approach as to that undertaken herein (Todd, McKnight et al. 2008) and likewise presented no associations with AD. Further, the LD structure for *BACE1* in this study is similar to that presented in both the prior study and in HapMap. Likewise, the calculated LD structure of *ADAM10* is very similar to that presented by HapMap. Whilst this study cannot categorically rule out the presence of an associated untyped variant, it appears unlikely due to the nature of the LD structures observed coupled with the number of SNPs analysed. As a power analysis revealed that, at a significance level of α=0.05, we had a power of 91% to detect a risk allele of 15% frequency mediating a relative risk of 1.6. In summary, this study, which represents the most thorough analysis of the *ADAM10* and *BACE1* genes, found no evidence for any involvement of these genes in AD. As such, this study, when combined with previously published data, suggests that common genetic variations in either *BACE1* or *ADAM10* are not associated with an increased risk to develop AD (Laws, Eckart et al. 2009). However, studies in much larger sample sizes would be required to categorically rule out their involvement. Another approach would be the investigation of epigenetic factors, pathway analysis or post-translational studies.

6.3.3 Summary and Discussion of Results for Genes Involved in Lipid Metabolism

6.3.3.1 Summary and Discussion of Results for *ABCA1*

The *ABCA1* gene represents a positional and functional candidate for AD risk. The location of *ABCA1* on chromosome 9q, which is approximately 5 Mb distal from previously identified loci linked with AD, makes the gene to a potential positional candidate gene (Pericak-Vance, Bass et al. 1998; Kehoe, Wavrant-De Vrieze et al. 1999; Bertram, Blacker et al. 2000; Myers, Wavrant De-Vrieze et al. 2002; Blacker, Bertram et al. 2003). The implication of ABCA1 in lipid metabolism and coronary artery disease (CAD), makes it also a reasonable functional candidate risk gene for AD. Moreover, it was shown that *ABCA1* expression can directly affect Aβ levels (Koldamova, Lefterov et al. 2003). In this study a whole fine mapping approach of *ABCA1* over the entire gene was performed. Verification of former findings, which were contradictory and achievement of new insights were the aim. Therefore, a total of 30 SNPs were analyzed covering the complete genetic region of *ABCA1* in a large case-control cohort. The initial single-marker, haplotype and sliding-window analyses revealed weak association signals for two markers in the 3'UTR region (SNP # 1, rs2525606 and # 2, rs2066720). Both SNPs underwent replication in a second large independent case-control series, including a pooled analysis, as well as an independent sib-pairs cohort. The formerly found associations were confirmed and suggest that the rs2525606-C allele and the rs2066720-G allele within the *ABCA1* gene are involved and contribute to the development of AD. For SNP # 1 the confidence interval (CI, 95%) for the odds ratio (OR = 1.53 for the C allele) was 0.9 – 2.3 in the initial Munich screening cohort. OR values below 1 indicate a protective function of the associated allele, whereas OR valaues above 1 indicate an increased disease risk associated with the allele. If CI of the OR spans 1 – the border between risk and protective – the results can not be regarded as trustworthy. Since SNP # 1 underwent replication and the former findings were confirmed with a higher and therefore better OR (2.08) and a reliable CI (1.4 – 3.0), C can be determined as risk allele.

Implications of the polymorphisms on *ABCA1* gene expression were tested and an effect was apparent for SNP # 3 (rs206676) located in an intron close to the 3'UTR of the gene. The risk allele (G) was associated with elevated *ABCA1* mRNA levels in the brain and seems to increase the risk for AD. This finding supports the formerly made observations by

Wahrle et al., where overexpression of *ABCA1* reduced amyloid deposition in a mouse model of Alzheimer´s disease, due to elevated ApoE lipidation (Wahrle, Jiang et al. 2008). Moreover it was shown by Koldamova and co-workers, that a lack of ABCA1 considerably increases amyloid deposition in APP23 mice, due to considerably decreased ApoE brain levels (Koldamova, Staufenbiel et al. 2005). These mechanisms are believed to include a role for ApoE in Aβ clearance (Schmitz, Kaminski et al. 2000; Canevari and Clark 2007; Hirsch-Reinshagen, Burgess et al. 2008). A possible reason could be a feed-back response that regulates ApoE levels and is triggered by plaque formation.

As already discussed for the *APP* results, the findings of the functional analyses have to be interpreted with caution, due to the small number of brain samples. Repetition of those experiments in a larger study cohort of brain samples is of utmost importance.

The two SNPs (# 1 and # 3) that yielded significant associations with AD in the initial Munich screening cohort as well as the Swedish replication were not analyzed and published by other groups up to now. 6 other SNPs from our thorough screening of the whole *ABCA1* gene locus found attention in literature before and were characterized with varying results. Most studies were carried out for the three exonic SNPs rs2230808, rs4149313 and rs2230806 (# 5, 9 and 16 in this study) (Wollmer, Streffer et al. 2003; Katzov, Chalmers et al. 2004; Li, Tacey et al. 2004; Kolsch, Lutjohann et al. 2006; Shibata, Kawarai et al. 2006; Chu, Li et al. 2007; Rodriguez-Rodriguez, Mateo et al. 2007; Wahrle, Shah et al. 2007; Wang and Jia 2007). The negative findings of Li, Rodriguez-Rodriguez, Shibata and Wahrle for the single marker associations were confirmed by the present study. In contrast, Katzov et al. found associations with AD for rs223080 and rs2230806 in one of the four examined collectives and Chu and Wang found an association with AD for rs2230808. Rodriguez-Rodriguez and co-workers had found an association signal for the haplotype generated by the three exonic SNPs rs2230808, rs4149313 and rs2230806, which could also not be confirmed by this study. Moreover, a correlation (D' and r^2) between the SNPs was not evident in this study, and therefore, the generation of a haplotype would not make sense. Due to the great distance between the different SNPs (24 and 34 kb) the results of Rodriguez-Rodriguez have to be taken with caution. Association results for intronic SNPs rs2777799 (# 4) and rs2575875 (# 23) were negative in the studies by Li et al. and Chu et al., respectively, which was confirmed by this study. SNP rs2066718 did not yield significant results in our Munich cohort of the present study, but had shown association with AD in one of the three studies by Li et al. and in one of the four studies by Katzov et al.

Different ethnic groups, size and composition of the cohorts and environmental influences - as already discussed before - are reasons for contradictive findings for the different study cohorts. With the exception of the studies from Chu and Wang, which were performed in Chinese subjects, the studied populations were similar and consisted all primarily of Caucasians with Northern European heritage. Hence, it seems to be most likely, that the examined SNPs only contribute a very small amount to the overall risk for AD.

In summary, this study is the largest and most comprehensive association analyses of the *ABCA1* gene, as a risk factor for sporadic AD, reported to date. In contrast to some of the prior studies, but in compliance with some other formerly published studies (Wollmer, Streffer et al. 2003; Katzov, Chalmers et al. 2004; Li, Tacey et al. 2004; Kolsch, Lutjohann et al. 2006; Shibata, Kawarai et al. 2006; Chu, Li et al. 2007; Rodriguez-Rodriguez, Mateo et al. 2007; Wahrle, Shah et al. 2007; Wang and Jia 2007) we observed no evidence for an association of exonic SNPs (rs2230808, rs4149313, rs2066718 and rs2230806) with sporadic AD. However, we found weak associations between the 3'UTR SNP rs2515606 and intronic rs2066720, which was replicated in additional sample sets, but was not discussed in the literature before.

6.3.3.2 Summary and Discussion of Results for *LIPC*

LIPC represents, due to its implication in lipid metabolism and coronary artery disease (CAD), a reasonable functional candidate risk gene for AD. Using a carefully ascertained case-control sample we performed a whole gene approach by genotyping of 25 SNPs that covered the entire genomic region of *LIPC* and determined the LD structure. Single marker as well as haplotype analysis did not reveal evidence of a significant association of common *LIPC* variants with sporadic forms of AD. Due to the fact that markers at *LIPC* show considerable variability in D' and r^2 values, and thus form several small LD blocks across the entire gene, it was important that multiple SNPs were investigated, as opposed to the *a priori* selection of relatively few SNPs as previously reported (Zhu, Taylor et al. 2008), so as to limit the likelihood of an untyped associated variant. Whilst this study cannot categorically rule out the presence of an associated untyped variant, it appears unlikely. Likewise with statistical power, as a power analysis revealed that, at a significance level of α=0.05, we had a power of 91% to detect a risk allele of 15% frequency mediating a relative risk of 1.6. In summary, this study combined with previously published data does not provide strong evidence that common genetic variations of *LIPC* are associated with an increased risk to develop AD. Our

genetic findings, however, do not rule out a possible functional involvement of *LIPC* in the pathogenesis of neurodegeneration in AD (Laws, Eckart et al. 2008).

6.4 Outlook

The association results from this work on hand and their positive replication in independent studies argue for an involvement of the selected SNPs from the *APP* and the *ABCA1* gene with the development of Alzheimer´s disease.

However, these findings can only be regarded as a first indication in view of the function of the identified candidate genes. Nevertheless, this study adds another piece to the complex puzzle of development of the multifactorial disease Morbus Alzheimer (AD).

Association signals within two marker peak regions spanning from intron 3 to 8 and intron 1, respectively in the *APP* gene were revealed for AD. According functional analyses are necessary to confirm the association results. Within this study we observed evidence to suggest that the identified SNPs have an impact on cognitive measures and both *APP* expression and processing. In the next step, the already applied functional approach should be repeated in a larger set of brain samples. It would also be worth looking for association with plaque formation, not only soluble Aß.

For *ABCA1* two SNPs had yielded significant association signals in our study cohorts. Since association signals were weak and previous studies from other laboratories had contradictive findings, the association study should be repeated in a larger collective to ensure association. Thereafter, a detailed functional approach, similar to the one applied for *APP*, should be performed to investigate the functional impact of the polymorphisms.

7 Appendix

7.1 Diagnostic Criteria for the Dementia Syndrome

7.1.1 ICD (International Classification of Diseases and Related Health Problems) 10 Criteria

Psychopathology:

- impairment of memory, especially in keeping new information, in severe cases also in remembering formerly learned information
- additional required impairments:

1. decline of other cognitive abilities (judgment, rationalness), both proven by medical history from third party as well as neuropsychological examination or quantified cognitive methods

2. decrease or affect control of initiative or social behaviour

- diagnosis is relied by presence of aphasia, apraxia or agnosia

Relevance for everyday life:

impairment of daily activities due to impairment of memory as well as due to loss of cognitive abilities

Duration:

impairment of memory and other coginitive abilities shall be present for at least 6 months

Criterion of exclusion:

- no impaired consciousness
- exclusion of other mental disorders (e.g. HIV-infection, vascular mental disorders, Morbus Parkinson, Chorea Huntington, normal pressure hydrocephalus), systemic diseases (e.g. hypothyroidism, vitamin-B12 or folic acid deficiency, hypercalemia) and alcohol or drug abuse

7.1.2 DSM IV (Diagnostic and Statisical Manual of Mental Disorders)

Psychopathology:

- impairment of memory (in keeping new information or remembering formerly learned information)
- additional required impairments:

  at least one of the following: aphasia, apraxia, agnosia, impairment of executive abilities (planning, organizing, sorting, abstracting)

Relevance for everyday life:

the cognitive disorders cause clear disorders in the social or job-related field and show a considerable impairment compared to the former niveau

Duration:

no determination

Criterion of exclusion:

deficits do not just occur during delirium

7.1.3 NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association) Criteria

<u>Possible AD</u>

- syndrome of dementia without neurological, psychiatric or systemic diseases, which could cause a dementia; also for different onset and clinical course
- additional presence of systemic or cerebral diseases, which could cause a dementia, but are not assumed to be the cause
- existence of a described, progressive, severe cognitive deficit, for which no other explanation is found

Probable AD

- clinical examination and documenatation of a dementia by means of MMSE, Blessed Dementia Scale or other tests
- deficit in at least two cognitive functions
- progressive impairment of memory and other cognitive functions
- no impaired consciousness
- onset between the age of 40 and 90, mostly however after the age of 65
- absence of systemic diseases or other diseases affecting the brain, which could explain the progressive loss of memory and cognition

Markers conformable with the disease:

- plateaus in progression of the disease
- associated symptoms like depression, insomnia, incontinence, hallucination, misperception, delution, overly verbal, emotional, physical reactions and sexual disruptions, progressive speech disorder
- other neurological abnormalities including symptoms in fine motor skills like elevated tonicity, myoclonia and apraxia
- epileptic seizure
- normal liquor, normal CT and EEG appropriate to age

Criterion of exclusion:

- sudden onset
- focal finding during physical neurological examination (e.g. hemisyndrome, coordination disorder at early stage)
- epileptic seizure or apraxia at early stage

Definitive AD

- presence of the clinical criteria for probable AD
- histopathological finding of biopsy or autopsy typical for AD

7.1.4 CERAD (Consortium to Establish a Registry for Alzheimer's Disease) Criteria

Normal with regard to AD and other dementia processes

- no anamnesis of dementia, no histopathological indication on AD and no other neuropathological lesions, which could cause a dementia
- an „A" age-based plaque score and no anamnesis of dementia
- anamnesis of dementia without neuropathological changes, which could cause a dementia

Possible AD

- an „A" age-based plaque score, anamnesis of dementia and presence or absence of other neuropathological lesions, which could cause a dementia
- a „B" or „C" age-based plaque score, but no clinical manifestation of dementia

Probable AD

- a „B" age-based plaque score, anamnesis of dementia and presence or absence of other neuropathological lesions, which could cause a dementia

Definitive AD

- a „C" age-based plaque score, anamnesis of dementia and presence or absence of other neuropathological lesions, which could cause a dementia

Age-based plaque score / Semiquantitative determination of the maximum density of neuritic plaques at hundredfold magnification:

Tab. 44: AD diagnostic according to CERAD criteria

Density of neuritic plaques is rated as moderate, intermediate or frequent. This density is converted into an age-based plaques score and reported as CERAD 0, A, B or C. (0 = no histological indication of AD, A = uncertain histological indication of AD, B = hist histological indication of AD and C = clear histological indication of AD).

age at death	Frequency of neuritc plaques			
	no plaques	moderate	intermediate	frequent
< 50	0	C	C	C
50 – 75	0	B	C	C
> 75	0	A	B	C

7.2 Statistics of the Used Study Cohorts

Tab. 45: Statistics and composition of the Munich study cohort, including sub-collectives

	MUC complete	MUC EOAD	MUC LOAD	MUC ApoE4	MUC nonApoE4
N (total)	728	281	444	295	433
N (controls)	290	139	149	64	226
Mean age (years)	66.8	56.6	76.3	67.1	66.7
SD (years)	12.0	6.9	6.6	11.8	12.0
% males	44.0	44.6	43.0	42.2	44.2
N (cases)	438	142	295	231	207
Mean age (years)	72.2	61.8	77.2	72.0	72.5
SD (years)	9.1	5.6	5.5	8.3	9.9
Mean age onset (years)	69.0	58.4	74.1	68.6	69.5
SD (years)	9.1	5.1	5.5	8.4	9.7
% males	41.0	51.4	35.9	39.4	42.5

Tab. 46: Statistics and composition of the replication study cohorts, including sub-collectives

	SWE	SWE EOAD	SWE LOAD	pooled	pooled EOAD	pooled LOAD	sibs
N (total)	883	220	661	1611	501	1105	735
N (controls)	631	126	504	921	265	653	438
Mean age (years)	73.1	60.0	76.4	71.2	58.2	76.4	68.4
SD (years)	8.9	0.0	6.7	10.4	5.3	6.7	10.1
% males	39.4	36.5	40.1	40.8	40.8	40.8	41.6
N (cases)	252	94	157	690	236	452	297
Mean age (years)	-	-	-	70.7	61.4	75.3	72.0
SD (years)	-	-	-	8.4	4.7	5.6	9.1
Mean age onset (years)	67.9	61.3	71.8	68.6	59.6	73.3	71.5
SD (years)	6.1	2.5	3.7	8.1	4.5	5.1	5.1
% males	40.0	39.4	40.5	40.6	46.6	37.4	41.6

Tab. 47: Statistics and composition of the cerebrospinal fluid samples

N (total)	85
Mean age (years)	66.7
SD (years)	9.5
Mean age onset (years)	66.0
SD (years)	9.3
% males	52.9

7.3 Results from Additional Statistical Tests Referred to but not Included in the Text

7.3.1 Additional Statistical Test Results for *APP*

Tab. 48: Results from genotypic tests for *APP* gene in the ApoE ε4/non ApoE ε4 subcollectives of the Munich study cohort

#	SNP	Genotypic test results	
		AD affection genotypic p-values for major allele unless otherwise noted (associated allele); OR (95%CI)with covariates (sex and onset)	
		ApoE ε4 subcollective (N = 295)	non*ApoE* ε4 subcollective (N = 433)
1	rs2040124	0.796	0.996
2	rs2829955	0.050 (G); 2.15 (1.01-4.6)	0.656
3	rs6516704	0.307	0.921
4	rs6516708	0.511	0.421
6	rs373521	0.404	0.770
7	rs2070653	0.150	0.767
8	rs2829975	0.071	0.143
9	rs1783016	0.922	0.645
10	rs2829983	0.982	0.893
11	rs216781	0.648	0.594
12	rs2186301	0.797	0.203
13	rs216762	0.684	0.365
14	rs762479	0.965	0.236
15	rs9978555	0.438	0.226
16	rs9941877	0.072	0.278
17	rs2830012	0.152	0.722
18	rs2830019	0.523	0.449
19	rs768039	0.766	0.067
20	rs3787644	0.130	0.017 (C); 1.61 (1.1-2.4)
21	rs2070655	0.847	0.094
22	rs3991	0.092	0.739
23	rs2830035	0.390	0.579
24	rs743532	0.109	0.925
25	rs1041420	0.122	0.682
26	rs2830046	0.207	0.576
27	rs8130594	0.540	0.544
28	rs2830053	0.854	0.184
29	rs2246115	0.960	0.132
30	rs2830069	0.627	0.154
31	rs462278	0.753	0.919
32	rs2830082	0.845	0.208
33	rs1788283	0.188	0.628
34	rs2830092	0.788	0.104
35	rs6516727	0.156	0.213
36	rs2830099	0.160	0.491
37	rs465984	0.732	0.981
38	rs1235885	0.189	0.674
39	rs456565	0.330	0.312

Tab. 49: Results from allelic tests for *APP* gene in the Swedish and pooled study cohorts

#	SWE (N=883)					pooled Population (MUC+SWE) (N=1611)				
	Associated allele	Major allele frequency (cases, controls)	Allelic p-values from Haploview			Associated allele	Major allele frequency (cases, controls)	Allelic p-values from Haploview		
			SWE complete (N=883)	EOAD subcollective (N=220)	LOAD subcollective (N=661)			pooled complete (N=1611)	EOAD subcollective (N=501)	LOAD subcollective (N=1105)
15	T	0.971, 0.958	0.215	0.755	0.077	C	0.959, 0.960	0.946	0.602	0.683
16	G	0.757, 0.731	0.283	0.384	0.435	A	0.711, 0.735	0.134	0.755	0.101
17	C	0.713, 0.693	0.481	0.652	0.618	T	0.681, 0.698	0.317	0.386	0.521
18	A	0.963, 0.955	0.673	0.782	0.380	A	0.959, 0.959	0.913	0.425	0.685
20	T	0.650, 0.666	0.549	0.183	0.090	C	0.666, 0.649	0.328	**0.011**	0.570
21	A	0.703, 0.709	0.812	**0.031**	0.055	C	0.709, 0.697	0.457	0.006	0.300
22	G	0.800, 0.798	0.920	0.852	0.862	G	0.803, 0.787	0.299	0.958	0.225
23	C	0.756, 0.748	0.761	0.311	0.518	C	0.760, 0.743	0.280	0.628	0.396
25	T	0.109, 0.747	0.131	0.259	0.295	T	0.722, 0.752	0.058	0.293	0.131
35	C	0.686, 0.695	0.728	0.607	0.287	T	0.703, 0.684	0.252	0.542	0.367
36	G	0.784, 0.735	**0.040**	0.077	0.393	G	0.755, 0.722	**0.036**	0.138	0.149

Tab. 50: Results from linear regression tests for *APP* gene - association between genotypes and Mini-Mental State Examination (MMSE)

#	SNP	Linear regression (association between genotype and MMSE score, using gender and onset as covariates)
1	rs2040124	0.397
2	rs2829955	0.275
3	rs6516704	0.467
4	rs6516708	0.350
5	rs11549660	0.171
6	rs373521	0.890
7	rs2070653	0.136
8	rs2829975	0.990
9	rs1783016	**0.018**
10	rs2829983	0.873
11	rs216781	0.979
12	rs2186301	0.442
13	rs216762	**0.011**
14	rs762479	0.175
15	rs9978555	0.676
16	rs9941877	0.828
17	rs2830012	0.734
18	rs2830019	0.934
19	rs768039	0.547
20	rs3787644	0.815
21	rs2070655	0.781
22	rs3991	0.856
23	rs2830035	0.800
24	rs743532	0.409
25	rs1041420	0.156
26	rs2830046	0.631
27	rs8130594	0.351
28	rs2830053	**0.008**
29	rs2246115	0.107
30	rs2830069	**0.011**
31	rs462278	0.342
32	rs2830082	**0.006**
33	rs1788283	0.153
34	rs2830092	**0.014**
35	rs6516727	0.744
36	rs2830099	0.797
37	rs465984	0.303
38	rs1235885	0.424
39	rs8131261	0.125

Tab. 51: Results from linear regression tests for *APP* gene - association between genotypes and age at onset

#	SNP	Linear regression (association between genotype and age at onset using gender as covariate)
1	rs2040124	0.447
2	rs2829955	0.897
3	rs6516704	0.653
4	rs6516708	0.705
5	rs11549660	0.392
6	rs373521	0.311
7	rs2070653	0.759
8	rs2829975	0.931
9	rs1783016	**0.032**
10	rs2829983	0.501
11	rs216781	0.148
12	rs2186301	0.270
13	rs216762	0.201
14	rs762479	0.296
15	rs9978555	0.273
16	rs9941877	0.925
17	rs2830012	0.981
18	rs2830019	0.799
19	rs768039	0.486
20	rs3787644	0.160
21	rs2070655	0.281
22	rs3991	0.094
23	rs2830035	0.275
24	rs743532	0.600
25	rs1041420	0.908
26	rs2830046	0.122
27	rs8130594	0.958
28	rs2830053	0.821
29	rs2246115	0.431
30	rs2830069	0.676
31	rs462278	0.837
32	rs2830082	0.907
33	rs1788283	0.161
34	rs2830092	0.782
35	rs6516727	0.362
36	rs2830099	0.242
37	rs465984	0.948
38	rs1235885	0.657
39	rs8131261	0.152

7.3.2 Additional Statistical Test Results for *BACE1*

Tab. 52: Results from allelic tests for *BACE1* gene in the sub-collectives of the Munich study cohort

#	SNP	EOAD subcollective (N=281)	LOAD subcollective (N=444)
1	rs644215	0.427	0.454
2	rs12292027	0.806	0.587
3	rs1047964	0.424	0.853
4	rs638405	0.230	0.166
5	rs507805	0.709	0.653
6	rs609332	0.954	0.268
7	rs522843	0.148	0.893
8	rs687740	0.071	0.769
9	rs473210	0.058	0.415
10	rs551662	0.799	0.771
11	rs525493	0.287	0.913

7.3.3 Additional Statistical Test Results for ADAM10

Tab. 53: Results from allelic tests for *ADAM10* gene in the sub-collectives of the Munich study cohort

#	SNP	EOAD subcollective (N=281)	LOAD subcollective (N=444)
1	rs12438487	0.712	0.862
2	rs1869135	0.663	0.516
3	rs7166076	0.693	0.434
4	rs7165035	0.820	0.440
5	rs2305421	0.672	0.413
6	rs12594872	0.713	0.982
7	rs1427282	0.723	0.954
8	rs7163733	0.691	0.510
9	rs7174386	0.641	0.330
10	rs9302203	0.612	0.564
11	rs6494031	0.959	0.485
12	rs11071392	0.670	0.300
13	rs8026668	0.606	0.846
14	rs11071393	0.641	0.260
15	rs1427280	0.928	0.528
16	rs1427281	0.455	0.332
17	rs4775086	0.822	0.644
18	rs2052805	0.482	0.693
19	rs2657125	0.309	0.971
20	rs347117	0.534	0.829
21	rs4238331	0.476	0.899
22	rs12441313	0.777	0.487
23	rs6494038	0.668	0.692
24	rs12439231	0.738	0.280
25	rs383902	0.284	0.525
26	rs653765	0.326	0.243
27	rs593742	0.270	0.189

7.3.4 Additional Statistical Test Results for ABCA1

Tab. 54: Results from genotypic tests for *ABCA1* gene in the ApoE ε4/non ApoE ε4 subcollectives of the Munich study cohort

#	SNP	Genotypic test results	
		AD affection genotypic p-values for major allele unless otherwise noted (associated allele); OR (95%CI)with covariates (sex and onset)	
		ApoE ε4 subcollective (N = 295)	non*ApoE* ε4 subcollective (N = 433)
1	rs2515606	0.846	0.050 (C); 1.75 (0.9-3.1)
2	rs1331924	0.260	0.648
3	rs2066720	0.045 (A); 2.73 (1.02-7.3)	0.763
4	rs2777799	0.880	0.550
5	rs2230808	0.080	0.968
6	rs2066716	0.196	0.866
7	rs2297401	0.654	0.992
8	rs3818689	0.086	0.470
9	rs4149313	0.984	0.079
10	rs2066718	0.859	0.397
11	rs2297398	0.600	0.238
12	rs2487062	0.388	0.966
13	rs914544	0.367	0.180
14	rs1175293	0.261	0.935
15	rs2472445	0.872	0.830
16	rs2230806	0.925	0.950
17	rs2230805	0.925	0.780
18	rs13301006	0.474	0.381
19	rs2000069	0.755	0.720
20	rs1999429	0.584	0.457
21	rs2275542	0.362	0.623
22	rs3847305	0.659	0.952
23	rs2575875	0.488	0.687
24	rs3758294	0.377	0.861
25	rs2487042	0.822	0.478
26	rs2487035	0.131	0.580
27	rs2472516	0.375	0.275
28	rs4397467	0.751	0.015 (G); 2.35 (1.2-4.7)
29	rs4743776	0.393	0.463
30	rs737623	0.758	0.104

Tab. 55: Results from allelic tests for *ABCA1* gene in the Swedish and pooled study cohorts

#	SWE (N=883)					pooled Population (MUC+SWE) (N=1611)				
	Associated allele	Major allele frequency (cases, controls)	Allelic p-values from Haploview			Associated allele	Major allele frequency (cases, controls)	Allelic p-values from Haploview		
			SWE complete (N=883)	EOAD subcollective (N=220)	LOAD subcollective (N=661)			pooled complete (N=1611)	EOAD subcollective (N=501)	LOAD subcollective (N=1105)
1	C	0.493, 0.432	**0.031**	**0.050**	0.189	C	0.447, 0.414	0.064	**0.023**	0.467
3	G	0.918, 0.876	**0.018**	**0.007**	0.225	G	0.895, 0.874	0.086	0.078	0.349

Tab. 56: Results from linear regression tests for *ABCA1* gene - association between genotypes and Mini-Mental State Examination (MMSE)

#	SNP	Linear regression (association between genotype and MMSE score, using gender and onset as covariates)
1	rs2515606	0.688
2	rs1331924	0.576
3	rs2066720	0.604
4	rs2777799	0.590
5	rs2230808	0.431
6	rs2066716	0.821
7	rs2297401	0.127
8	rs3818689	0.958
9	rs4149313	0.788
10	rs2066718	0.879
11	rs2297398	0.727
12	rs2487062	0.845
13	rs914544	0.115
14	rs1175293	0.810
15	rs2472445	0.360
16	rs2230806	0.787
17	rs2230805	0.920
18	rs13301006	0.148
19	rs2000069	0.133
20	rs1999429	0.327
21	rs2275542	0.216
22	rs3847305	0.110
23	rs2575875	0.367
24	rs3758294	0.762
25	rs2487042	0.358
26	rs2487035	0.465
27	rs2472516	0.755
28	rs4397467	0.355
29	rs4743776	0.941
30	rs737623	0.807

Tab. 57: Results from linear regression tests for *ABCA1* gene - association between genotypes and age at onset

#	SNP	Linear regression (association between genotype and age at onset using gender as covariate)
1	rs2515606	0.332
2	rs1331924	0.790
3	rs2066720	0.642
4	rs2777799	0.531
5	rs2230808	0.762
6	rs2066716	0.337
7	rs2297401	0.913
8	rs3818689	0.812
9	rs4149313	0.861
10	rs2066718	0.756
11	rs2297398	0.628
12	rs2487062	0.131
13	rs914544	0.681
14	rs1175293	0.729
15	rs2472445	0.502
16	rs2230806	0.814
17	rs2230805	0.436
18	rs13301006	0.206
19	rs2000069	0.106
20	rs1999429	0.447
21	rs2275542	0.908
22	rs3847305	0.885
23	rs2575875	0.731
24	rs3758294	0.180
25	rs2487042	0.739
26	rs2487035	0.969
27	rs2472516	0.271
28	rs4397467	0.462
29	rs4743776	0.399
30	rs737623	0.162

7.3.5 Additional Statistical Test Results for *LIPC*

Tab. 58: Results from allelic tests for *LIPC* gene in the sub-collectives of the Munich study cohort

#	SNP	EOAD subcollective (N=281)	LOAD subcollective (N=444)
1	rs4774297	0.324	0.324
2	rs1800588	0.143	0.143
3	rs17190517	0.109	0.109
4	rs261341	0.600	0.600
5	rs483140	0.308	0.308
6	rs261336	0.129	0.129
7	rs3825776	0.931	0.931
8	rs10518976	0.217	0.217
9	rs10518978	0.404	0.404
10	rs6494011	0.700	0.700
11	rs1968685	0.940	0.940
12	rs1869137	0.803	0.803
13	rs1973028	0.632	0.632
14	rs2899631	0.280	0.280
15	rs4774302	0.776	0.776
16	rs2899632	0.413	0.413
17	rs12899928	0.556	0.556
18	rs4775072	0.411	0.411
19	rs6078	0.785	0.785
20	rs2242064	0.900	0.900
21	rs871804	0.776	0.776
22	rs2414594	0.811	0.811
23	rs3829462	0.803	0.803
24	rs6074	0.830	0.830
25	rs4774308	0.903	0.903

7.4 Results from Sliding Window Analyses

Note that significant p-values (≤0.05) are reported in **bold** font. Haplotypes with frequencies < 5 % are not reported and were not taken into account for the graphs, due to their unlikely occurrence.

7.4.1 Results from Sliding Window Analyses for *APP*

Tab. 59: Results from sliding window analysis of 2-marker haplotypes for *APP* – complete Munich cohort

Window	Marker	Haplotype ID	Sequence	Frequency	p-values
1	1-2	1.1	CC	0.519	0.6829
		1.2	CG	0.469	0.5926
2	2-3	2.1	CA	0.525	0.6166
		2.2	GT	0.465	0.6780
3	3-4	3.1	AC	0.516	0.5101
		3.2	TA	0.464	0.6495
4	4-5	4.1	CC	0.515	0.4801
		4.2	AC	0.484	0.5350
5	5-6	5.1	CG	0.568	0.9145
		5.2	CT	0.431	0.8459
6	6-7	6.1	TT	0.405	0.7805
		6.2	GT	0.345	0.7840
		6.3	GC	0.224	0.5833
7	7-8	7.1	TA	0.631	0.2631
		7.2	CA	0.250	0.4758
		7.3	TC	0.119	0.4752
8	8-9	8.1	AG	0.638	0.6667
		8.2	AA	0.243	0.3013
		8.3	CG	0.110	0.4913
9	9-10	9.1	GA	0.635	0.7432
		9.2	AA	0.252	0.3323
		9.3	GC	0.113	0.4050
10	10-11	10.1	AA	0.854	0.6672
		10.2	CA	0.078	0.7650
11	11-12	11.1	AC	0.906	0.7101
		11.2	GC	0.068	0.3355
12	12-13	12.1	CG	0.505	0.5446
		12.2	CA	0.469	0.7363
13	13-14	13.1	AC	0.442	0.4106
		13.2	GC	0.344	0.6046
		13.3	GG	0.186	0.3275
14	14-15	14.1	CT	0.784	0.1301
		14.2	GT	0.174	0.2549
15	15-16	15.1	TG	0.701	**0.0096**
		15.2	TA	0.256	**0.0216**
16	16-17	16.1	GC	0.653	**0.0446**
		16.2	AT	0.254	**0.0482**
		16.3	GT	0.060	0.7345
17	17-18	17.1	CA	0.686	0.0942
		17.2	TA	0.278	0.2010
18	18-19	18.1	AC	0.644	0.1263
		18.2	AT	0.318	0.0618
19	19-20	19.1	CC	0.643	**0.0168**
		19.2	TT	0.313	0.0613
20	20-21	20.1	CC	0.648	**0.0186**

		20.2	TA	0.309	0.0857
21	21-22	21.1	CG	0.524	**0.0144**
		21.2	AG	0.264	0.2669
		21.3	CT	0.172	0.2091
22	22-23	22.1	GC	0.744	**0.0470**
		22.2	TT	0.206	0.1447
23	23-24	23.1	CG	0.702	0.0892
		23.2	TG	0.250	0.1965
24	24-25	24.1	GC	0.695	0.3300
		24.2	GT	0.257	0.1270
25	25-26	25.1	CT	0.499	0.8353
		25.2	TT	0.253	0.1736
		25.3	CC	0.244	0.0771
26	26-27	26.1	TG	0.445	0.2119
		26.2	TC	0.307	0.9139
		26.3	CG	0.244	0.1175
27	27-28	27.1	GG	0.614	0.5769
		27.2	CG	0.288	0.9946
		27.3	GA	0.076	0.3791
28	28-29	28.1	GC	0.857	0.0845
		28.2	AG	0.096	0.5880
29	29-30	29.1	CA	0.861	0.1127
		29.2	GC	0.097	0.4446
30	30-31	30.1	AT	0.877	0.2521
		30.2	CT	0.097	0.3606
31	31-32	31.1	TC	0.875	0.2258
		31.2	TT	0.098	0.3259
32	32-33	32.1	CG	0.555	0.3904
		32.2	CT	0.347	0.1307
		32.3	TT	0.097	0.3617
33	33-34	33.1	GG	0.556	0.3942
		33.2	TG	0.347	0.1107
		33.3	TT	0.097	0.2569
34	34-35	34.1	GT	0.595	0.1923
		34.2	GC	0.308	**0.0326**
		34.3	TT	0.095	0.2620
35	35-36	35.1	TG	0.690	**0.0298**
		35.2	CC	0.275	0.0712
36	36-37	36.1	GG	0.696	0.0898
		36.2	CG	0.278	**0.0497**
37	37-38	37.1	GA	0.961	0.7698
38	38-39	38.1	AT	0.561	0.4478
		38.2	AC	0.402	0.3671

Tab. 60: Results from sliding window analysis of 3-marker haplotypes for *APP* – complete Munich cohort

Window	Marker	Haplotype ID	Sequence	Frequency	p-values
1	1-3	1.1	CCA	0.514	0.6606
		1.2	CGT	0.464	0.6313
2	2-4	2.1	CAC	0.517	0.5118
		2.2	GTA	0.462	0.7233
3	3-5	3.1	ACC	0.516	0.5101
		3.2	TAC	0.462	0.7124
4	4-6	4.1	ACG	0.471	0.6203
		4.2	CCT	0.426	0.7603
		4.3	CCG	0.096	0.5811
5	5-7	5.1	CTT	0.405	0.8079
		5.2	CGT	0.345	0.7731
		5.3	CGC	0.224	0.6027
6	6-8	6.1	TTA	0.397	0.9140
		6.2	GTA	0.234	0.3005
		6.3	GCA	0.223	0.5376
		6.4	GTC	0.112	0.3769
7	7-9	7.1	TAG	0.500	0.8401
		7.2	CAG	0.137	0.7227
		7.3	TAA	0.131	0.0613
		7.4	CAA	0.113	0.5785
		7.5	TCG	0.111	0.5259
8	8-10	8.1	AGA	0.528	0.9411
		8.2	AAA	0.242	0.2932
		8.3	AGC	0.110	0.4368
		8.4	CGA	0.107	0.5190
9	9-11	9.1	GAA	0.631	0.6780
		9.2	AAA	0.223	0.4444
		9.3	GCA	0.078	0.7242
10	10-12	10.1	AAC	0.829	0.9487
		10.2	CAC	0.078	0.7838
11	11-13	11.1	ACG	0.474	0.6550
		11.2	ACA	0.432	0.4964
12	12-14	12.1	CAC	0.442	0.3924
		12.2	CGC	0.318	0.8315
		12.3	CGG	0.187	0.3273
13	13-15	13.1	ACT	0.439	0.3731
		13.2	GCT	0.346	0.6846
		13.3	GGT	0.173	0.2802
14	14-16	14.1	CTG	0.637	**0.0057**
		14.2	CTA	0.147	0.0568
		14.3	GTA	0.109	0.2528
		14.4	GTG	0.065	0.6625
15	15-17	15.1	TGC	0.645	**0.0306**
		15.2	TAT	0.221	0.0523
		15.3	TGT	0.058	0.6013
16	16-18	16.1	GCA	0.651	0.0613
		16.2	ATA	0.218	0.1151
		16.3	GTA	0.060	0.7377
17	17-19	17.1	CAC	0.373	0.7170
		17.2	CAT	0.313	**0.0409**
		17.3	TAC	0.272	0.2410
18	18-20	18.1	ACC	0.605	**0.0395**
		18.2	ATT	0.313	0.0615

19	19-21	19.1	CCC	0.641	**0.0158**
		19.2	TTA	0.303	0.0650
20	20-22	20.1	CCG	0.480	**0.0028**
		20.2	TAG	0.268	0.2190
		20.3	CCT	0.168	0.3273
21	21-23	21.1	CGC	0.483	**0.0119**
		21.2	AGC	0.261	0.3744
		21.3	CTT	0.167	0.4016
22	22-24	22.1	GCG	0.696	**0.0185**
		22.2	TTG	0.206	0.1446
23	23-25	23.1	CGC	0.445	0.8225
		23.2	CGT	0.256	0.1166
		23.3	TGC	0.250	0.2065
24	24-26	24.1	GCT	0.452	0.5332
		24.2	GTT	0.253	0.1783
		24.3	GCC	0.242	0.0837
25	25-27	25.1	CTG	0.439	0.3058
		25.2	TTC	0.249	0.3021
		25.3	CCG	0.241	0.0981
		25.4	CTC	0.061	0.1622
26	26-28	26.1	TGG	0.369	0.4554
		26.2	TCG	0.286	0.8893
		26.3	CGG	0.242	0.1014
		26.4	TGA	0.076	0.3592
27	27-29	27.1	GGC	0.574	0.1912
		27.2	CGC	0.283	0.9287
		27.3	GAG	0.073	0.5482
28	28-30	28.1	GCA	0.857	0.0859
		28.2	AGC	0.094	0.5044
29	29-31	29.1	CAT	0.834	0.0790
		29.2	GCT	0.097	0.4446
30	30-32	30.1	ATC	0.875	0.2229
		30.2	CTT	0.096	0.3315
31	31-33	31.1	TCG	0.554	0.3891
		31.2	TCT	0.321	0.0735
		31.3	TTT	0.097	0.3608
32	32-34	32.1	CGG	0.556	0.3648
		32.2	CTG	0.347	0.1314
		32.3	TTT	0.097	0.3641
33	33-35	33.1	GGT	0.561	0.3590
		33.2	TGC	0.305	**0.0442**
		33.3	TTT	0.095	0.2532
34	34-36	34.1	GTG	0.596	0.2040
		34.2	GCC	0.275	0.0710
		34.3	TTG	0.094	0.1915
35	35-37	35.1	TGG	0.664	0.0559
		35.2	CCG	0.275	0.0712
36	36-38	36.1	GGA	0.690	0.0867
		36.2	CGA	0.271	0.0563
37	37-39	37.1	GAT	0.559	0.4303
		37.2	GAC	0.402	0.3637

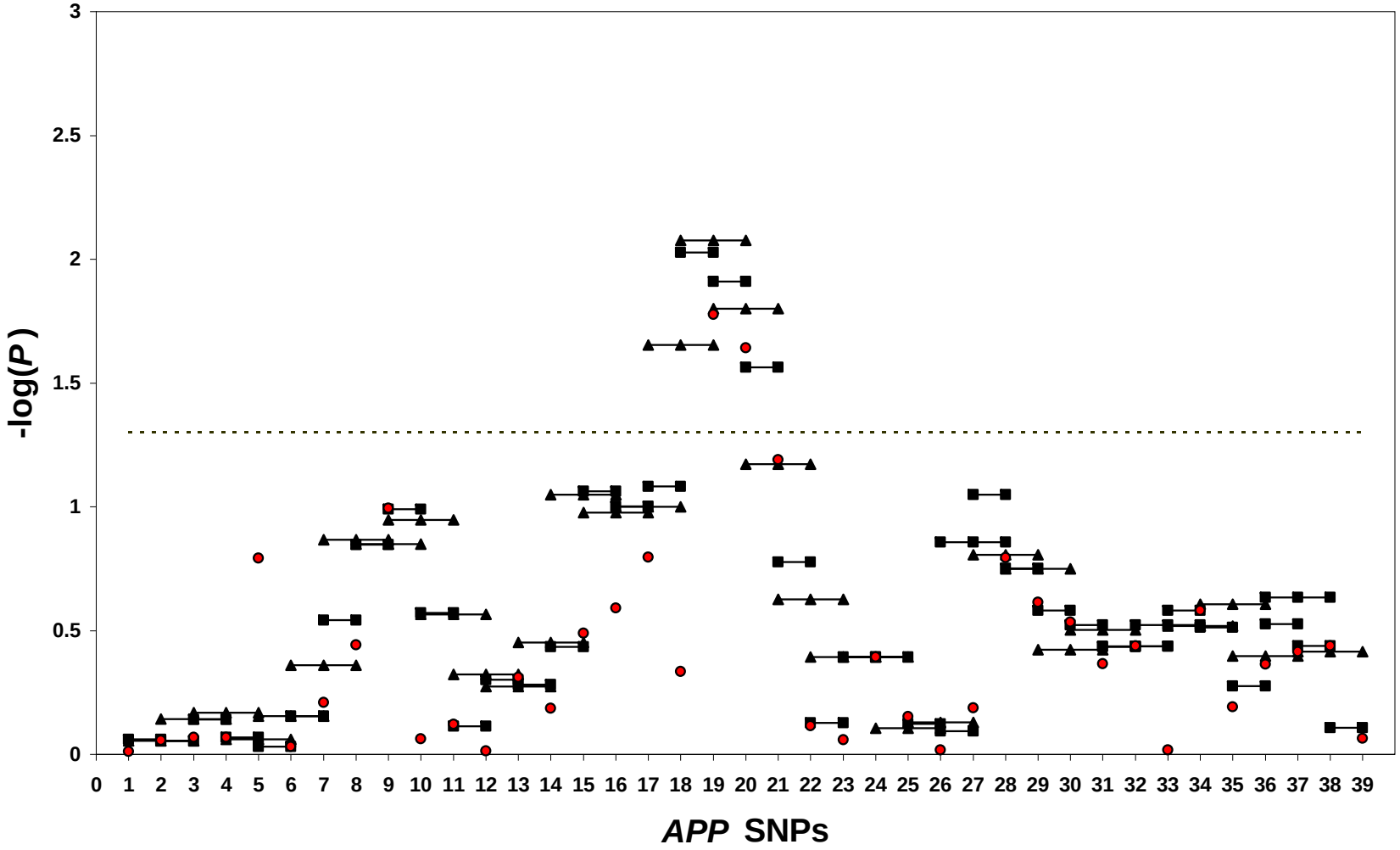

Fig. 33: Sliding window analysis of 2- and 3-marker haplotypes for *APP* – Munich EOAD subcollective

Tab. 61: Results from sliding window analysis of 2-marker haplotypes for *APP* – Munich EOAD subcollective

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-2	1.1	CC	0.535	0.8705
		1.2	CG	0.450	0.8796
2	2-3	2.1	CA	0.544	0.9529
		2.2	GT	0.446	0.8832
3	3-4	3.1	AC	0.527	0.7228
		3.2	TA	0.443	0.8061
4	4-5	4.1	CC	0.526	0.8556
		4.2	AC	0.471	0.9881
5	5-6	5.1	CG	0.567	0.9323
		5.2	CT	0.429	0.9325
6	6-7	6.1	TT	0.390	0.6994
		6.2	GT	0.364	0.9369
		6.3	GC	0.207	0.9912
7	7-8	7.1	TA	0.632	0.2876
		7.2	CA	0.247	0.6146
		7.3	TC	0.121	0.3647
8	8-9	8.1	AG	0.614	0.4742
		8.2	AA	0.265	0.1424
		8.3	CG	0.114	0.2341
9	9-10	9.1	GA	0.622	0.1116
		9.2	AA	0.272	0.1022
		9.3	GC	0.107	0.8854
10	10-11	10.1	AA	0.856	0.4952
		10.2	CA	0.082	0.2682
11	11-12	11.1	AC	0.916	0.8104
		11.2	GC	0.062	0.7683
12	12-13	12.1	CG	0.504	0.5005
		12.2	CA	0.474	0.5025
13	13-14	13.1	AC	0.454	0.5230
		13.2	GC	0.335	0.7735
		13.3	GG	0.191	0.7073
14	14-15	14.1	CT	0.784	0.7992
		14.2	GT	0.167	0.3670
15	15-16	15.1	TG	0.717	0.2562
		15.2	TA	0.234	0.0866
16	16-17	16.1	GC	0.665	0.0995
		16.2	AT	0.239	0.4510
		16.3	GT	0.065	0.2345
17	17-18	17.1	CA	0.690	0.1649
		17.2	TA	0.275	0.0829
18	18-19	18.1	AC	0.652	**0.0094**
		18.2	AT	0.313	**0.0170**
19	19-20	19.1	CC	0.654	**0.0123**
		19.2	TT	0.309	**0.0267**
20	20-21	20.1	CC	0.659	**0.0273**
		20.2	TA	0.293	0.0505
21	21-22	21.1	CG	0.532	0.1672
		21.2	AG	0.256	0.1907
		21.3	CT	0.176	0.7116
22	22-23	22.1	GC	0.756	0.7450
		22.2	TT	0.202	0.9034
23	23-24	23.1	CG	0.714	0.7997
		23.2	TG	0.237	0.8754

24	24-25	24.1	GC	0.673	0.9639
		24.2	GT	0.277	0.7206
25	25-26	25.1	CT	0.477	0.7530
		25.2	TT	0.274	0.7654
		25.3	CC	0.246	0.9650
26	26-27	26.1	TG	0.428	0.8040
		26.2	TC	0.323	0.7195
		26.3	CG	0.246	0.8413
27	27-28	27.1	GG	0.579	0.5690
		27.2	CG	0.308	0.7259
		27.3	GA	0.095	0.0892
28	28-29	28.1	GC	0.856	0.1774
		28.2	AG	0.112	0.3144
29	29-30	29.1	CA	0.860	0.2619
		29.2	GC	0.110	0.3775
30	30-31	30.1	AT	0.869	0.5454
		30.2	CT	0.107	0.2999
31	31-32	31.1	TC	0.869	0.6283
		31.2	TT	0.106	0.3670
32	32-33	32.1	CG	0.558	0.9853
		32.2	CT	0.336	0.5685
		32.3	TT	0.106	0.3661
33	33-34	33.1	GG	0.557	0.9517
		33.2	TG	0.335	0.5011
		33.3	TT	0.108	0.2620
34	34-35	34.1	GT	0.591	0.8473
		34.2	GC	0.302	0.5741
		34.3	TT	0.103	0.3070
35	35-36	35.1	TG	0.693	0.6042
		35.2	CC	0.272	0.5288
36	36-37	36.1	GG	0.700	0.2969
		36.2	CG	0.277	0.4379
37	37-38	37.1	GA	0.960	0.3650
38	38-39	38.1	AT	0.562	0.7815
		38.2	AC	0.397	0.9323

Tab. 62: Results from sliding window analysis of 3-marker haplotypes for *APP* – Munich EOAD subcollective

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-3	1.1	CCA	0.530	0.9435
		1.2	CGT	0.446	0.8832
2	2-4	2.1	CAC	0.527	0.7194
		2.2	GTA	0.443	0.7533
3	3-5	3.1	ACC	0.527	0.7228
		3.2	TAC	0.440	0.6789
4	4-6	4.1	ACG	0.457	0.9297
		4.2	CCT	0.423	0.8694
		4.3	CCG	0.112	0.8854
5	5-7	5.1	CTT	0.390	0.7006
		5.2	CGT	0.363	0.9925
		5.3	CGC	0.205	0.9273
6	6-8	6.1	TTA	0.381	0.6053
		6.2	GTA	0.253	0.6679
		6.3	GCA	0.206	0.9674
		6.4	GTC	0.112	0.4360
7	7-9	7.1	TAG	0.489	0.9190
		7.2	TAA	0.144	0.1356
		7.3	CAG	0.124	0.3407
		7.4	CAA	0.121	0.6738
		7.5	TCG	0.114	0.2497
8	8-10	8.1	AGA	0.506	0.4414
		8.2	AAA	0.266	0.1416
		8.3	CGA	0.115	0.2264
		8.4	AGC	0.107	0.8975
9	9-11	9.1	GAA	0.618	0.1131
		9.2	AAA	0.239	0.2442
		9.3	GCA	0.081	0.2528
10	10-12	10.1	AAC	0.837	0.5655
		10.2	CAC	0.080	0.2717
11	11-13	11.1	ACG	0.476	0.5751
		11.2	ACA	0.440	0.4764
12	12-14	12.1	CAC	0.455	0.5317
		12.2	CGC	0.312	0.7788
		12.3	CGG	0.192	0.7092
13	13-15	13.1	ACT	0.448	0.6020
		13.2	GCT	0.336	0.7675
		13.3	GGT	0.168	0.3536
14	14-16	14.1	CTG	0.652	0.1360
		14.2	CTA	0.131	0.0895
		14.3	GTA	0.103	0.5159
		14.4	GTG	0.066	0.4926
15	15-17	15.1	TGC	0.655	0.1054
		15.2	TAT	0.202	0.1686
		15.3	TGT	0.063	0.2676
16	16-18	16.1	GCA	0.665	0.0998
		16.2	ATA	0.207	0.3169
		16.3	GTA	0.063	0.1654
17	17-19	17.1	CAC	0.382	0.4060
		17.2	CAT	0.309	**0.0222**
		17.3	TAC	0.271	0.0619
18	18-20	18.1	ACC	0.620	**0.0084**
		18.2	ATT	0.309	**0.0272**

19	19-21	19.1	CCC	0.652	**0.0158**
		19.2	TTA	0.292	**0.0309**
20	20-22	20.1	CCG	0.488	0.0672
		20.2	TAG	0.257	0.1386
		20.3	CCT	0.171	0.7172
21	21-23	21.1	CGC	0.506	0.2370
		21.2	AGC	0.250	0.2970
		21.3	CTT	0.166	0.5623
22	22-24	22.1	GCG	0.706	0.4812
		22.2	TTG	0.202	0.9034
23	23-25	23.1	CGC	0.437	0.8366
		23.2	CGT	0.273	0.6480
		23.3	TGC	0.236	0.7786
24	24-26	24.1	GCT	0.429	0.9991
		24.2	GTT	0.274	0.7845
		24.3	GCC	0.244	0.9684
25	25-27	25.1	CTG	0.415	0.9923
		25.2	TTC	0.266	0.9635
		25.3	CCG	0.240	0.8931
		25.4	CTC	0.065	0.7434
26	26-28	26.1	TGG	0.334	0.5229
		26.2	TCG	0.307	0.7698
		26.3	CGG	0.244	0.9330
		26.4	TGA	0.095	0.1387
27	27-29	27.1	GGC	0.554	0.3661
		27.2	CGC	0.302	0.9544
		27.3	GAG	0.094	0.1563
28	28-30	28.1	GCA	0.856	0.1778
		28.2	AGC	0.108	0.3038
29	29-31	29.1	CAT	0.836	0.4609
		29.2	GCT	0.110	0.3775
30	30-32	30.1	ATC	0.869	0.5636
		30.2	CTT	0.107	0.3147
31	31-33	31.1	TCG	0.555	0.9758
		31.2	TCT	0.314	0.6924
		31.3	TTT	0.106	0.3663
32	32-34	32.1	CGG	0.557	0.9762
		32.2	CTG	0.336	0.5181
		32.3	TTT	0.107	0.3009
33	33-35	33.1	GGT	0.562	0.9128
		33.2	TGC	0.298	0.5221
		33.3	TTT	0.101	0.3034
34	34-36	34.1	GTG	0.590	0.8201
		34.2	GCC	0.272	0.5275
		34.3	TTG	0.103	0.2472
35	35-37	35.1	TGG	0.669	0.4353
		35.2	CCG	0.272	0.5288
36	36-38	36.1	GGA	0.691	0.2322
		36.2	CGA	0.269	0.3960
37	37-39	37.1	GAT	0.561	0.7760
		37.2	GAC	0.398	0.9384

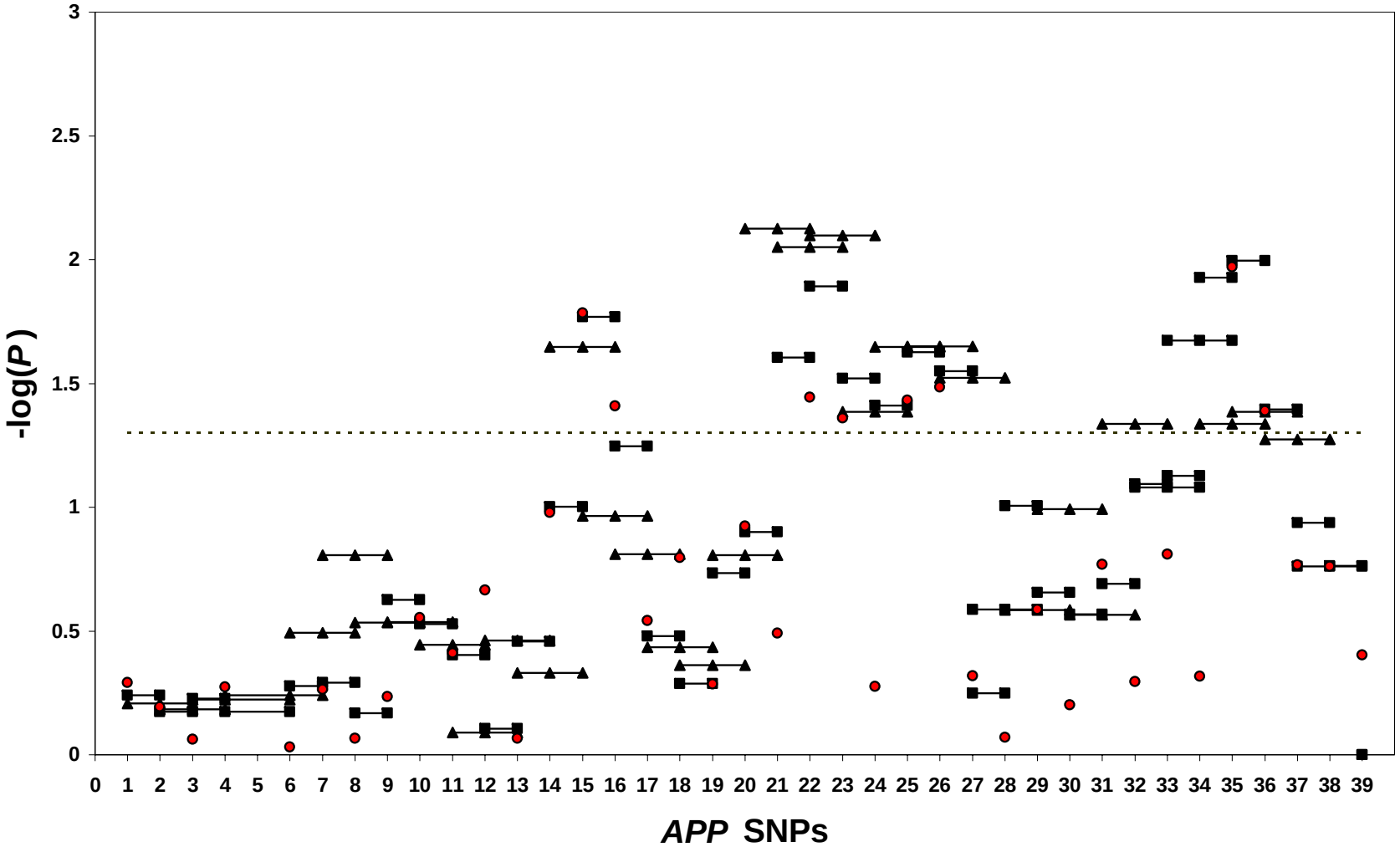

Fig. 34: Sliding window analysis of 2- and 3-marker haplotypes for *APP* – Munich LOAD subcollective

Tab. 63: Results from sliding window analysis of 2-marker haplotypes for *APP* – Munich LOAD subcollective

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-2	1.1	CC	0.507	0.6681
		1.2	CG	0.482	0.5757
2	2-3	2.1	CA	0.510	0.6690
		2.2	GT	0.479	0.6888
3	3-4	3.1	AC	0.507	0.6990
		3.2	TA	0.479	0.5929
4	4-6	4.1	AG	0.483	0.7130
		4.2	CT	0.426	0.8515
		4.3	CG	0.086	0.6706
5	6-7	5.1	TT	0.412	0.9188
		5.2	GT	0.334	0.6506
		5.3	GC	0.235	0.5269
6	7-8	6.1	TA	0.630	0.5100
		6.2	CA	0.252	0.5424
		6.3	TC	0.119	0.8681
7	8-9	7.1	AG	0.658	0.6787
		7.2	AA	0.223	0.7420
		7.3	CG	0.106	0.9105
8	9-10	8.1	GA	0.647	0.2363
		8.2	AA	0.236	0.5827
		8.3	GC	0.117	0.3012
9	10-11	9.1	AA	0.852	0.2960
		9.2	CA	0.076	0.5470
10	11-12	10.1	AC	0.900	0.9471
		10.2	GC	0.072	0.3945
11	12-13	11.1	CG	0.503	0.7853
		11.2	CA	0.468	0.8906
12	13-14	12.1	AC	0.437	0.6365
		12.2	GC	0.347	0.3602
		12.3	GG	0.184	0.3490
13	14-15	13.1	CT	0.782	0.0997
		13.2	GT	0.179	0.5404
14	15-16	14.1	TG	0.691	**0.0170**
		14.2	TA	0.270	0.1454
15	16-17	15.1	GC	0.645	0.2213
		15.2	AT	0.262	0.0568
		15.3	GT	0.057	0.1555
16	17-18	16.1	CA	0.681	0.3318
		16.2	TA	0.279	0.6901
17	18-19	17.1	AC	0.640	0.9475
		17.2	AT	0.320	0.5164
18	19-20	18.1	CC	0.637	0.1845
		18.2	TT	0.314	0.4335
19	20-21	19.1	CC	0.642	0.1259
		19.2	TA	0.317	0.3380
20	21-22	20.1	CG	0.520	**0.0248**
		20.2	AG	0.267	0.5483
		20.3	CT	0.170	0.0606
21	22-23	21.1	GC	0.736	**0.0128**
		21.2	TT	0.209	0.0651
		21.3	GT	0.053	0.2604
22	23-24	22.1	CG	0.694	**0.0301**
		22.2	TG	0.259	**0.0479**

23	24-25	23.1	GC	0.708	0.0970
		23.2	GT	0.245	**0.0389**
24	25-26	24.1	CT	0.512	0.8300
		24.2	CC	0.243	**0.0236**
		24.3	TT	0.240	0.0560
25	26-27	25.1	TG	0.454	0.2239
		25.2	TC	0.298	0.5076
		25.3	CG	0.244	**0.0282**
26	27-28	26.1	GG	0.634	0.5648
		26.2	CG	0.275	0.6168
		26.3	GA	0.064	0.8613
27	28-29	27.1	GC	0.856	0.2580
		27.2	AG	0.087	0.9353
		27.3	GG	0.053	0.0985
28	29-30	28.1	CA	0.860	0.2565
		28.2	GC	0.090	0.6568
29	30-31	29.1	AT	0.881	0.2705
		29.2	CT	0.091	0.6352
30	31-32	30.1	TC	0.879	0.2038
		30.2	TT	0.093	0.5071
31	32-33	31.1	CG	0.550	0.1954
		31.2	CT	0.357	0.0804
		31.3	TT	0.091	0.5632
32	33-34	32.1	GG	0.552	0.1903
		32.2	TG	0.357	0.0747
		32.3	TT	0.091	0.4822
33	34-35	33.1	GT	0.595	0.0505
		33.2	GC	0.314	**0.0118**
		33.3	TT	0.091	0.4676
34	35-36	34.1	TG	0.687	**0.0101**
		34.2	CC	0.279	**0.0460**
35	36-37	35.1	GG	0.692	0.1313
		35.2	CG	0.281	**0.0402**
36	37-38	36.1	GA	0.962	0.2322
37	38-39	37.1	AT	0.558	0.3988
		37.2	AC	0.406	0.1723

Tab. 64: Results from sliding window analysis of 3-marker haplotypes for *APP* – Munich LOAD subcollective

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-3	1.1	CCA	0.501	0.6960
		1.2	CGT	0.477	0.6210
2	2-4	2.1	CAC	0.508	0.7022
		2.2	GTA	0.477	0.6536
3	3-6	3.1	TAG	0.477	0.6528
		3.2	ACT	0.421	0.9282
		3.3	ACG	0.086	0.5983
4	4-7	4.1	CTT	0.408	0.7955
		4.2	AGT	0.251	0.9352
		4.3	AGC	0.231	0.5758
		4.4	CGT	0.082	0.6401
5	6-8	5.1	TTA	0.406	0.8503
		5.2	GCA	0.234	0.4584
		5.3	GTA	0.223	0.3217
		5.4	GTC	0.112	0.6333
6	7-9	6.1	TAG	0.512	0.9523
		6.2	CAG	0.145	0.6254
		6.3	TAA	0.122	0.3883
		6.4	TCG	0.108	0.9189
		6.5	CAA	0.103	0.1564
7	8-10	7.1	AGA	0.545	0.2918
		7.2	AAA	0.222	0.7425
		7.3	AGC	0.114	0.3081
		7.4	CGA	0.102	0.9028
8	9-11	8.1	GAA	0.642	0.2904
		8.2	AAA	0.210	0.6970
		8.3	GCA	0.077	0.5966
9	10-12	9.1	AAC	0.824	0.6397
		9.2	CAC	0.076	0.5373
10	11-13	10.1	ACG	0.470	0.8431
		10.2	ACA	0.429	0.8118
11	12-14	11.1	CAC	0.437	0.6073
		11.2	CGC	0.318	0.6419
		11.3	CGG	0.185	0.3459
12	13-15	12.1	ACT	0.436	0.4673
		12.2	GCT	0.347	0.4910
		12.3	GGT	0.177	0.5879
13	14-16	13.1	CTG	0.626	**0.0225**
		13.2	CTA	0.155	0.2648
		13.3	GTA	0.114	0.4180
		13.4	GTG	0.065	0.9980
14	15-17	14.1	TGC	0.638	0.1416
		14.2	TAT	0.232	0.1940
		14.3	TGT	0.055	0.1086
15	16-18	15.1	GCA	0.643	0.2595
		15.2	ATA	0.223	0.2118
		15.3	GTA	0.057	0.1546
16	17-19	16.1	CAC	0.367	0.9687
		16.2	CAT	0.314	0.3680
		16.3	TAC	0.272	0.8859
17	18-20	17.1	ACC	0.597	0.4545
		17.2	ATT	0.314	0.4335
18	19-21	18.1	CCC	0.636	0.1562

		18.2	TTA	0.309	0.3704
19	20-22	19.1	CCG	0.476	**0.0075**
		19.2	TAG	0.273	0.5322
		19.3	CCT	0.166	0.1077
20	21-23	20.1	CGC	0.470	**0.0089**
		20.2	AGC	0.266	0.6332
		20.3	CTT	0.167	0.1192
21	22-24	21.1	GCG	0.688	**0.0080**
		21.2	TTG	0.209	0.0650
		21.3	GTG	0.052	0.2652
22	23-25	22.1	CGC	0.448	0.7389
		22.2	TGC	0.259	**0.0411**
		22.3	CGT	0.246	**0.0452**
23	24-26	23.1	GCT	0.465	0.6187
		23.2	GCC	0.242	**0.0225**
		23.3	GTT	0.241	0.0568
24	25-27	24.1	CTG	0.453	0.3043
		24.2	CCG	0.242	**0.0224**
		24.3	TTC	0.238	0.0868
		24.4	CTC	0.058	0.1235
25	26-28	25.1	TGG	0.388	0.2425
		25.2	TCG	0.275	0.5273
		25.3	CGG	0.244	**0.0300**
		25.4	TGA	0.065	0.9475
26	27-29	26.1	GGC	0.585	0.2590
		26.2	CGC	0.272	0.7172
		26.3	GAG	0.061	0.7904
27	28-30	27.1	GCA	0.857	0.2597
		27.2	AGC	0.086	0.8455
28	29-31	28.1	CAT	0.832	0.1019
		28.2	GCT	0.090	0.6568
29	30-32	29.1	ATC	0.878	0.2724
		29.2	CTT	0.089	0.5231
30	31-33	30.1	TCG	0.550	0.2126
		30.2	TCT	0.328	**0.0269**
		30.3	TTT	0.091	0.5613
31	32-34	31.1	CGG	0.551	0.1939
		31.2	CTG	0.356	0.0832
		31.3	TTT	0.091	0.5791
32	33-35	32.1	GGT	0.557	0.1492
		32.2	TGC	0.312	**0.0212**
		32.3	TTT	0.091	0.4677
33	34-36	33.1	GTG	0.596	0.0567
		33.2	GCC	0.280	**0.0461**
		33.3	TTG	0.090	0.3688
34	35-37	34.1	TGG	0.659	**0.0411**
		34.2	CCG	0.279	**0.0460**
35	36-38	35.1	GGA	0.687	0.1708
		35.2	CGA	0.275	0.0532
36	37-39	36.1	GAT	0.555	0.3734
		36.2	GAC	0.406	0.1729

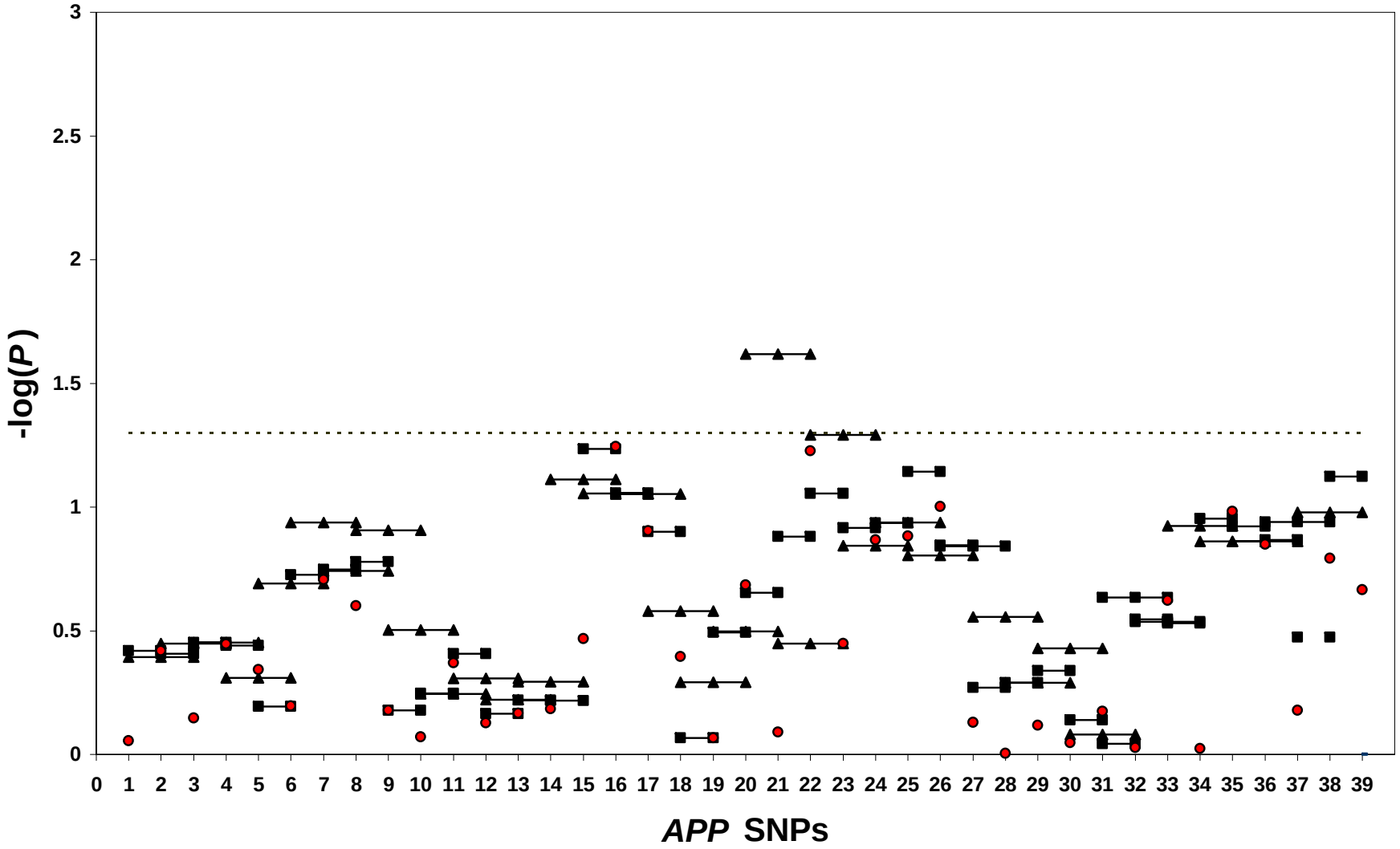

Fig. 35: Sliding window analysis of 2- and 3-marker haplotypes for *APP* – Munich ApoE ε4 subcollective

Tab. 65: Results from sliding window analysis of 2-marker haplotypes for *APP* – Munich ApoE ε4 subcollective

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-2	1.1	CC	0.513	0.3943
		1.2	CG	0.480	0.3809
2	2-3	2.1	CA	0.513	0.3916
		2.2	GT	0.476	0.4353
3	3-4	3.1	AC	0.502	0.3534
		3.2	TA	0.472	0.4810
4	4-5	4.1	CC	0.504	0.3629
		4.2	AC	0.493	0.4105
5	5-6	5.1	CG	0.562	0.7053
		5.2	CT	0.434	0.6409
6	6-7	6.1	TT	0.399	0.6793
		6.2	GT	0.347	0.5307
		6.3	GC	0.219	0.1876
7	7-8	7.1	TA	0.618	0.6847
		7.2	CA	0.253	0.1785
		7.3	TC	0.129	0.2466
8	8-9	8.1	AG	0.637	0.1936
		8.2	AA	0.235	0.5606
		8.3	CG	0.112	0.1664
9	9-10	9.1	GA	0.618	0.8012
		9.2	AA	0.251	0.6628
		9.3	GC	0.132	0.8436
10	10-11	10.1	AA	0.837	0.9043
		10.2	CA	0.091	0.5664
11	11-12	11.1	AC	0.909	0.3925
		11.2	GC	0.072	0.4342
12	12-13	12.1	CG	0.529	0.7559
		12.2	CA	0.451	0.6861
13	13-14	13.1	AC	0.416	0.6042
		13.2	GC	0.351	0.8742
		13.3	GG	0.199	0.7733
14	14-15	14.1	CT	0.764	0.6076
		14.2	GT	0.198	0.9036
15	15-16	15.1	TG	0.689	0.0582
		15.2	TA	0.273	0.1149
16	16-17	16.1	GC	0.635	0.0878
		16.2	AT	0.275	0.0951
		16.3	GT	0.056	0.9154
17	17-18	17.1	CA	0.669	0.1257
		17.2	TA	0.295	0.2166
18	18-19	18.1	AC	0.657	0.8790
		18.2	AT	0.306	0.8595
19	19-20	19.1	CC	0.644	0.3218
		19.2	TT	0.299	0.7110
20	20-21	20.1	CC	0.653	0.2216
		20.2	TA	0.298	0.8682
21	21-22	21.1	CG	0.563	0.2006
		21.2	AG	0.245	0.8026
		21.3	CT	0.144	0.1318
22	22-23	22.1	GC	0.762	0.1825
		22.2	TT	0.190	0.0881
23	23-24	23.1	CG	0.719	0.1212
		23.2	TG	0.235	0.3645

24	24-25	24.1	GC	0.684	0.4066
		24.2	GT	0.270	0.1159
25	25-26	25.1	CT	0.504	0.8661
		25.2	TT	0.269	0.1702
		25.3	CC	0.220	0.0719
26	26-27	26.1	TG	0.455	0.4134
		26.2	TC	0.318	0.5444
		26.3	CG	0.224	0.1427
27	27-28	27.1	GG	0.610	0.5593
		27.2	CG	0.294	0.5361
		27.3	GA	0.068	0.5952
28	28-29	28.1	GC	0.850	0.7326
		28.2	AG	0.095	0.8719
		28.3	GG	0.053	0.5141
29	29-30	29.1	CA	0.851	0.7760
		29.2	GC	0.093	0.8151
		29.3	GA	0.055	0.4587
30	30-31	30.1	AT	0.881	0.9347
		30.2	CT	0.087	0.7246
31	31-32	31.1	TC	0.877	0.9040
		31.2	TT	0.091	0.9092
32	32-33	32.1	CG	0.560	0.3290
		32.2	CT	0.345	0.2847
		32.3	TT	0.093	0.9929
33	33-34	33.1	GG	0.562	0.2975
		33.2	TG	0.344	0.2945
		33.3	TT	0.094	0.9483
34	34-35	34.1	GT	0.609	0.1297
		34.2	GC	0.297	0.1116
		34.3	TT	0.094	0.9665
35	35-36	35.1	TG	0.703	0.1197
		35.2	CC	0.262	0.1377
36	36-37	36.1	GG	0.709	0.1993
		36.2	CG	0.261	0.1356
37	37-38	37.1	GA	0.962	0.3365
38	38-39	38.1	AT	0.580	0.2196
		38.2	AC	0.383	0.0752

Tab. 66: Results from sliding window analysis of 3-marker haplotypes for *APP* – Munich ApoE ε4 subcollective

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-3	1.1	CCA	0.506	0.4050
		1.2	CGT	0.476	0.4354
2	2-4	2.1	CAC	0.503	0.3559
		2.2	GTA	0.470	0.5163
3	3-5	3.1	ACC	0.502	0.3534
		3.2	TAC	0.469	0.5368
4	4-6	4.1	ACG	0.480	0.4902
		4.2	CCT	0.431	0.4908
		4.3	CCG	0.082	0.7386
5	5-7	5.1	CTT	0.399	0.6795
		5.2	CGT	0.345	0.5002
		5.3	CGC	0.217	0.2042
6	6-8	6.1	TTA	0.392	0.9271
		6.2	GTA	0.227	0.8091
		6.3	GCA	0.216	0.1157
		6.4	GTC	0.122	0.3556
7	7-9	7.1	TAG	0.504	0.7287
		7.2	CAG	0.131	0.1813
		7.3	CAA	0.120	0.7253
		7.4	TAA	0.117	0.2689
		7.5	TCG	0.114	0.1665
8	8-10	8.1	AGA	0.510	0.2338
		8.2	AAA	0.235	0.5303
		8.3	AGC	0.127	0.8978
		8.4	CGA	0.107	0.1239
9	9-11	9.1	GAA	0.613	0.6717
		9.2	AAA	0.226	0.5651
		9.3	GCA	0.090	0.5619
10	10-12	10.1	AAC	0.818	0.8188
		10.2	CAC	0.091	0.5688
11	11-13	11.1	ACG	0.504	0.8736
		11.2	ACA	0.404	0.4927
12	12-14	12.1	CAC	0.417	0.6021
		12.2	CGC	0.330	0.9490
		12.3	CGG	0.200	0.7711
13	13-15	13.1	ACT	0.414	0.5096
		13.2	GCT	0.351	0.8129
		13.3	GGT	0.195	0.9776
14	14-16	14.1	CTG	0.618	0.0788
		14.2	CTA	0.145	0.0775
		14.3	GTA	0.127	0.7987
		14.4	GTG	0.072	0.9379
15	15-17	15.1	TGC	0.635	0.0881
		15.2	TAT	0.236	0.1764
		15.3	TGT	0.056	0.9128
16	16-18	16.1	GCA	0.635	0.0885
		16.2	ATA	0.238	0.1669
		16.3	GTA	0.056	0.9096
17	17-19	17.1	CAC	0.371	0.2630
		17.2	CAT	0.298	0.7344
		17.3	TAC	0.286	0.3001
18	18-20	18.1	ACC	0.608	0.5117
		18.2	ATT	0.299	0.7110

19	19-21	19.1	CCC	0.645	0.3177
		19.2	TTA	0.288	0.8158
20	20-22	20.1	CCG	0.510	**0.0241**
		20.2	TAG	0.249	0.7815
		20.3	CCT	0.143	0.1229
21	21-23	21.1	CGC	0.515	0.3559
		21.2	AGC	0.247	0.7997
		21.3	CTT	0.143	0.1908
22	22-24	22.1	GCG	0.716	0.0512
		22.2	TTG	0.190	0.0881
23	23-25	23.1	CGC	0.441	0.8921
		23.2	CGT	0.276	0.1433
		23.3	TGC	0.237	0.3353
24	24-26	24.1	GCT	0.459	0.5364
		24.2	GTT	0.269	0.1744
		24.3	GCC	0.219	0.1155
25	25-27	25.1	CTG	0.451	0.4473
		25.2	TTC	0.264	0.1756
		25.3	CCG	0.220	0.1572
		25.4	CTC	0.054	0.1729
26	26-28	26.1	TGG	0.382	0.5473
		26.2	TCG	0.294	0.3817
		26.3	CGG	0.225	0.1443
		26.4	TGA	0.072	0.6635
27	27-29	27.1	GGC	0.565	0.2776
		27.2	CGC	0.285	0.3569
		27.3	GAG	0.068	0.7311
28	28-30	28.1	GCA	0.850	0.7326
		28.2	AGC	0.093	0.8135
		28.3	GGA	0.053	0.5142
29	29-31	29.1	CAT	0.826	0.6923
		29.2	GCT	0.089	0.6617
		29.3	GAT	0.054	0.3725
30	30-32	30.1	ATC	0.878	0.9159
		30.2	CTT	0.089	0.8314
31	31-33	31.1	TCG	0.559	0.3591
		31.2	TCT	0.314	0.2326
		31.3	TTT	0.093	0.9932
32	32-34	32.1	CGG	0.560	0.3351
		32.2	CTG	0.344	0.2909
		32.3	TTT	0.094	0.9985
33	33-35	33.1	GGT	0.569	0.2748
		33.2	TGC	0.296	0.1193
		33.3	TTT	0.094	0.9665
34	34-36	34.1	GTG	0.610	0.1392
		34.2	GCC	0.262	0.1377
		34.3	TTG	0.094	0.9669
35	35-37	35.1	TGG	0.676	0.1998
		35.2	CCG	0.262	0.1379
36	36-38	36.1	GGA	0.703	0.2668
		36.2	CGA	0.259	0.1152
37	37-39	37.1	GAT	0.580	0.2196
		37.2	GAC	0.381	0.1052

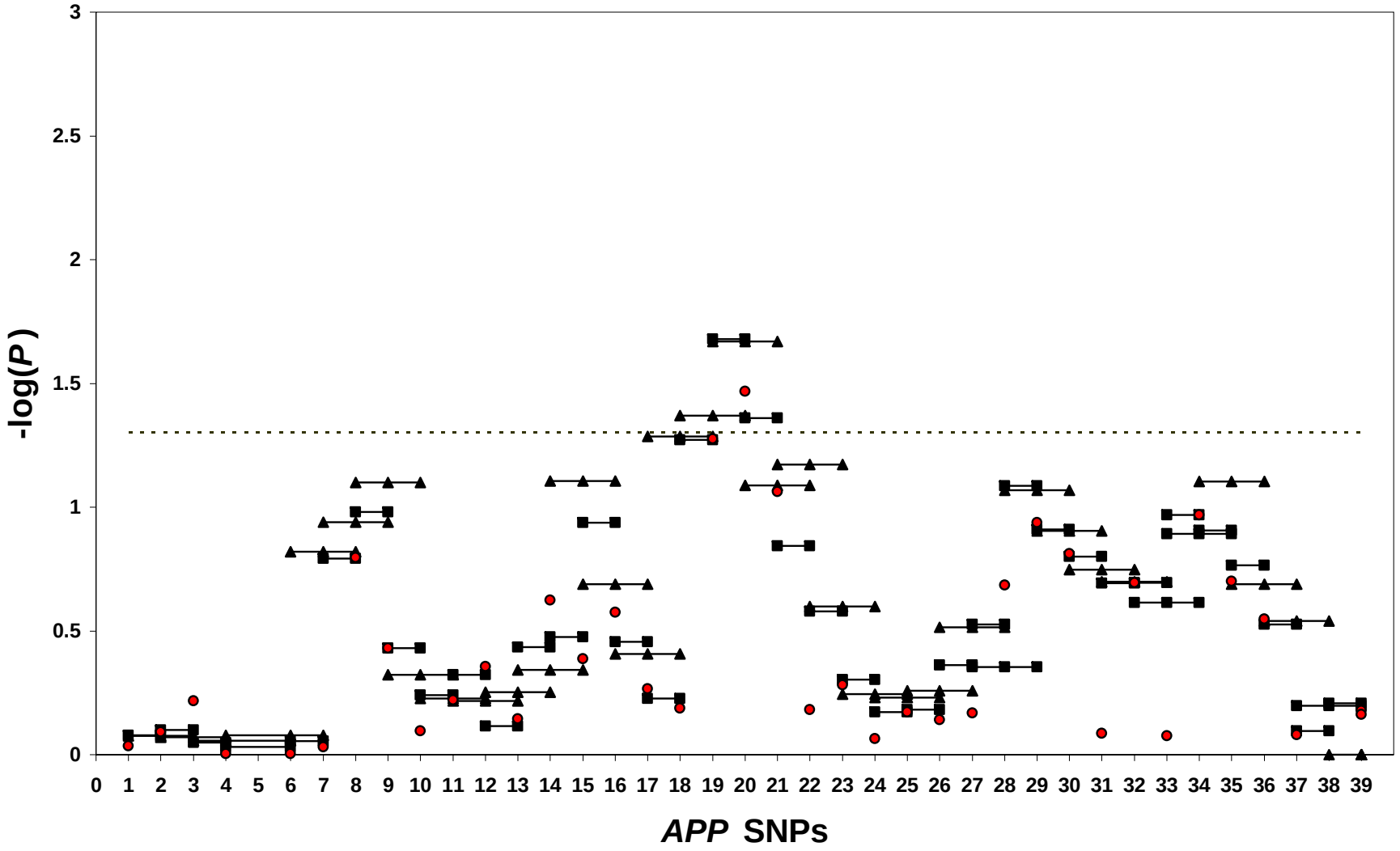

Fig. 36: Sliding window analysis of 2- and 3-marker haplotypes for *APP* – Munich nonApoE ε4 subcollective

Tab. 67: Results from sliding window analysis of 2-marker haplotypes for *APP* – Munich nonApoE ε4 subcollective

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-2	1.1	CC	0.523	0.8342
		1.2	CG	0.461	0.8529
2	2-3	2.1	CA	0.532	0.8241
		2.2	GT	0.458	0.7959
3	3-4	3.1	AC	0.526	0.8946
		3.2	TA	0.458	0.8991
4	4-6	4.1	AG	0.466	0.9760
		4.2	CT	0.423	0.9681
		4.3	CG	0.106	0.9307
5	6-7	5.1	TT	0.407	0.9782
		5.2	GT	0.345	0.9004
		5.3	GC	0.227	0.8817
6	7-8	6.1	TA	0.639	0.4078
		6.2	CA	0.248	0.9153
		6.3	TC	0.113	0.1610
7	8-9	7.1	AG	0.640	0.8232
		7.2	AA	0.247	0.4306
		7.3	CG	0.107	0.1045
8	9-10	8.1	GA	0.647	0.5016
		8.2	AA	0.253	0.3703
		8.3	GC	0.100	0.8200
9	10-11	9.1	AA	0.866	0.9110
		9.2	CA	0.069	0.5733
10	11-12	10.1	AC	0.907	0.8794
		10.2	GC	0.064	0.4765
11	12-13	11.1	CG	0.488	0.9734
		11.2	CA	0.482	0.7675
12	13-14	12.1	AC	0.460	0.9143
		12.2	GC	0.340	0.3671
		12.3	GG	0.177	0.5591
13	14-15	13.1	CT	0.797	0.3344
		13.2	GT	0.157	0.5073
14	15-16	14.1	TG	0.709	0.1156
		14.2	TA	0.245	0.2045
15	16-17	15.1	GC	0.664	0.3500
		15.2	AT	0.240	0.4330
		15.3	GT	0.062	0.8432
16	17-18	16.1	CA	0.695	0.5939
		16.2	TA	0.266	0.7207
17	18-19	17.1	AC	0.635	0.0890
		17.2	AT	0.326	0.0535
18	19-20	18.1	CC	0.642	**0.0209**
		18.2	TT	0.323	0.0803
19	20-21	19.1	CC	0.644	**0.0437**
		19.2	TA	0.316	0.0727
20	21-22	20.1	CG	0.495	0.1434
		20.2	AG	0.279	0.2203
		20.3	CT	0.193	0.9784
21	22-23	21.1	GC	0.732	0.2633
		21.2	TT	0.218	0.8273
22	23-24	22.1	CG	0.690	0.4969
		22.2	TG	0.260	0.5288
23	24-25	23.1	GC	0.705	0.7524

		23.2	GT	0.245	0.6728
24	25-26	24.1	CT	0.495	0.9419
		24.2	CC	0.260	0.6565
		24.3	TT	0.242	0.7730
25	26-27	25.1	TG	0.438	0.4344
		25.2	TC	0.300	0.5783
		25.3	CG	0.258	0.6548
26	27-28	26.1	GG	0.615	0.8261
		26.2	CG	0.284	0.5487
		26.3	GA	0.082	0.2974
27	28-29	27.1	GC	0.862	0.0819
		27.2	AG	0.097	0.4096
28	29-30	28.1	CA	0.867	0.1232
		28.2	GC	0.100	0.2100
29	30-31	29.1	AT	0.877	0.1643
		29.2	CT	0.100	0.1584
30	31-32	30.1	TC	0.877	0.2038
		30.2	TT	0.099	0.2031
31	32-33	31.1	CG	0.552	0.7539
		31.2	CT	0.349	0.2597
		31.3	TT	0.099	0.2020
32	33-34	32.1	GG	0.552	0.8228
		32.2	TG	0.349	0.2133
		32.3	TT	0.100	0.1075
33	34-35	33.1	GT	0.586	0.7873
		33.2	GC	0.315	0.1831
		33.3	TT	0.096	0.1244
34	35-36	34.1	TG	0.682	0.1721
		34.2	CC	0.285	0.3617
35	36-37	35.1	GG	0.687	0.3427
		35.2	CG	0.289	0.2971
36	37-38	36.1	GA	0.961	0.8030
37	38-39	37.1	AT	0.547	0.7446
		37.2	AC	0.415	0.6210

Tab. 68: Results from sliding window analysis of 3-marker haplotypes for *APP* – Munich nonApoE ε4 subcollective

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-3	1.1	CCA	0.518	0.8498
		1.2	CGT	0.456	0.8401
2	2-4	2.1	CAC	0.526	0.8935
		2.2	GTA	0.457	0.8506
3	3-6	3.1	TAG	0.455	0.8987
		3.2	ACT	0.419	0.9715
		3.3	ACG	0.107	0.8769
4	4-7	4.1	CTT	0.404	0.8349
		4.2	AGT	0.245	0.8895
		4.3	AGC	0.220	0.8829
		4.4	CGT	0.100	0.9382
5	6-8	5.1	TTA	0.400	0.9900
		5.2	GTA	0.239	0.3795
		5.3	GCA	0.227	0.8719
		5.4	GTC	0.105	0.1514
6	7-9	6.1	TAG	0.499	0.9598
		6.2	TAA	0.140	0.2312

		6.3	CAG	0.140	0.7398
		6.4	TCG	0.108	0.1151
		6.5	CAA	0.107	0.8423
7	8-10	7.1	AGA	0.540	0.6752
		7.2	AAA	0.246	0.4666
		7.3	CGA	0.106	0.0795
		7.4	AGC	0.100	0.8279
		8.1	GAA	0.648	0.4763
8	9-11	8.2	AAA	0.216	0.4772
		8.3	GCA	0.070	0.5768
9	10-12	9.1	AAC	0.837	0.7446
		9.2	CAC	0.069	0.5917
10	11-13	10.1	ACG	0.455	0.8274
		10.2	ACA	0.452	0.8988
11	12-14	11.1	CAC	0.459	0.8682
		11.2	CGC	0.310	0.5615
		11.3	CGG	0.178	0.5601
12	13-15	12.1	ACT	0.455	0.9174
		12.2	GCT	0.342	0.4549
		12.3	GGT	0.157	0.4904
13	14-16	13.1	CTG	0.649	0.0783
		13.2	CTA	0.148	0.2306
		13.3	GTA	0.096	0.5712
		13.4	GTG	0.062	0.6755
14	15-17	14.1	TGC	0.651	0.2045
		14.2	TAT	0.210	0.3246
		14.3	TGT	0.059	0.6398
15	16-18	15.1	GCA	0.663	0.3920
		15.2	ATA	0.204	0.6525
		15.3	GTA	0.061	0.9321
16	17-19	16.1	CAC	0.373	0.1805
		16.2	CAT	0.323	0.0517
		16.3	TAC	0.262	0.6861
17	18-20	17.1	ACC	0.604	**0.0427**
		17.2	ATT	0.323	0.0812
18	19-21	18.1	CCC	0.639	**0.0214**
		18.2	TTA	0.313	0.0736
19	20-22	19.1	CCG	0.458	0.0818
		19.2	TAG	0.283	0.1601
		19.3	CCT	0.186	0.7886
20	21-23	20.1	CGC	0.458	0.0673
		20.2	AGC	0.273	0.3512
		20.3	CTT	0.185	0.7601
21	22-24	21.1	GCG	0.682	0.2523
		21.2	TTG	0.218	0.8272
22	23-25	22.1	CGC	0.447	0.8048
		22.2	TGC	0.259	0.5685
		22.3	CGT	0.242	0.6478
23	24-26	23.1	GCT	0.447	0.8163
		23.2	GCC	0.258	0.5876
		23.3	GTT	0.243	0.7714
24	25-27	24.1	CTG	0.432	0.5947
		24.2	CCG	0.254	0.5528
		24.3	TTC	0.239	0.9799
		24.4	CTC	0.066	0.5816
25	26-28	25.1	TGG	0.359	0.8365
		25.2	TCG	0.282	0.5159
		25.3	CGG	0.256	0.6064
		25.4	TGA	0.079	0.3057
26	27-29	26.1	GGC	0.578	0.4424
		26.2	CGC	0.284	0.6172
		26.3	GAG	0.077	0.4781

27	28-30	27.1	GCA	0.862	0.0853
		27.2	AGC	0.095	0.3022
28	29-31	28.1	CAT	0.843	0.1248
		28.2	GCT	0.100	0.2100
29	30-32	29.1	ATC	0.876	0.2199
		29.2	CTT	0.097	0.1788
30	31-33	30.1	TCG	0.551	0.7242
		30.2	TCT	0.326	0.2006
		30.3	TTT	0.099	0.2016
31	32-34	31.1	CGG	0.552	0.7539
		31.2	CTG	0.349	0.2430
		31.3	TTT	0.099	0.1806
32	33-35	32.1	GGT	0.555	0.8344
		32.2	TGC	0.312	0.2148
		32.3	TTT	0.096	0.1279
33	34-36	33.1	GTG	0.587	0.8058
		33.2	GCC	0.285	0.3614
		33.3	TTG	0.095	0.0787
34	35-37	34.1	TGG	0.658	0.2047
		34.2	CCG	0.285	0.3617
35	36-38	35.1	GGA	0.681	0.2879
		35.2	CGA	0.280	0.3193
36	37-39	36.1	GAT	0.546	0.7071
		36.2	GAC	0.415	0.6345

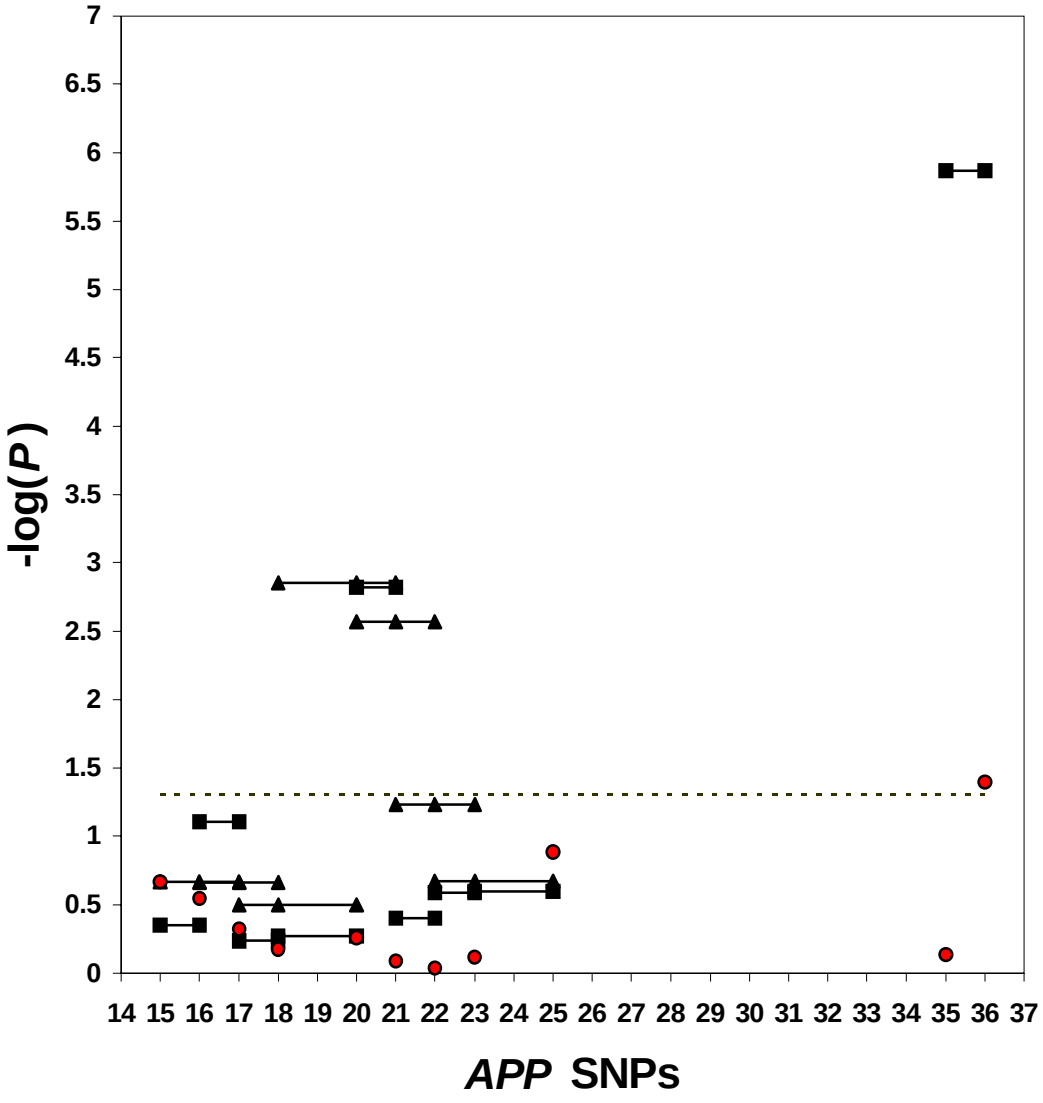

Fig. 37: Sliding window analysis of 2- and 3-marker haplotypes for *APP* – Swedish study cohort

Tab. 69: Results from sliding window analysis of 2-marker haplotypes for *APP* – Swedish study cohort

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	15-16	1.1	TG	0.727	0.4463
		1.2	TA	0.235	0.7944
2	16-17	2.1	GC	0.660	0.8463
		2.2	AT	0.228	0.0786
		2.3	GT	0.076	0.2203
3	17-18	3.1	CA	0.693	0.5801
		3.2	TA	0.262	0.7313
4	18-20	4.1	AC	0.620	0.6947

		4.2	AT	0.336	0.5412
5	20-21	5.1	CC	0.644	0.0617
		5.2	TA	0.276	0.2853
		5.3	TC	0.062	**0.0015**
6	21-22	6.1	CG	0.551	0.6850
		6.2	AG	0.248	0.7157
		6.3	CT	0.156	0.3985
7	22-23	7.1	GC	0.739	0.6104
		7.2	TT	0.189	0.2211
		7.3	GT	0.060	0.2573
8	23-25	8.1	CC	0.496	0.4486
		8.2	CT	0.254	0.2525
		8.3	TC	0.241	0.6246
9	35-36	9.1	TG	0.685	0.4230
		9.2	CC	0.246	**0.0165**
		9.3	CG	0.060	**1.35E-06**

Tab. 70: Results from sliding window analysis of 3-marker haplotypes for *APP* – Swedish study cohort

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	15-17	1.1	TGC	0.649	0.8961
		1.2	TAT	0.202	0.3731
		1.3	TGT	0.075	0.2156
2	16-18	2.1	GCA	0.659	0.9040
		2.2	ATA	0.190	0.2166
		2.3	GTA	0.073	0.2411
3	17-20	3.1	CAC	0.373	0.5186
		3.2	CAT	0.319	0.8148
		3.3	TAC	0.248	0.3171
4	18-21	4.1	ACC	0.607	0.1192
		4.2	ATA	0.274	0.2955
		4.3	ATC	0.062	**0.0014**
5	20-22	5.1	CCG	0.490	0.3053
		5.2	TAG	0.230	0.0777
		5.3	CCT	0.154	0.2990
		5.4	TCG	0.060	**0.0027**
6	21-23	6.1	CGC	0.492	0.9283
		6.2	AGC	0.246	0.5290
		6.3	CTT	0.147	0.0586
		6.4	CGT	0.057	0.4037
7	22-25	7.1	GCC	0.488	0.2333
		7.2	GCT	0.250	0.3916
		7.3	TTC	0.186	0.2116
		7.4	GTC	0.054	0.3916

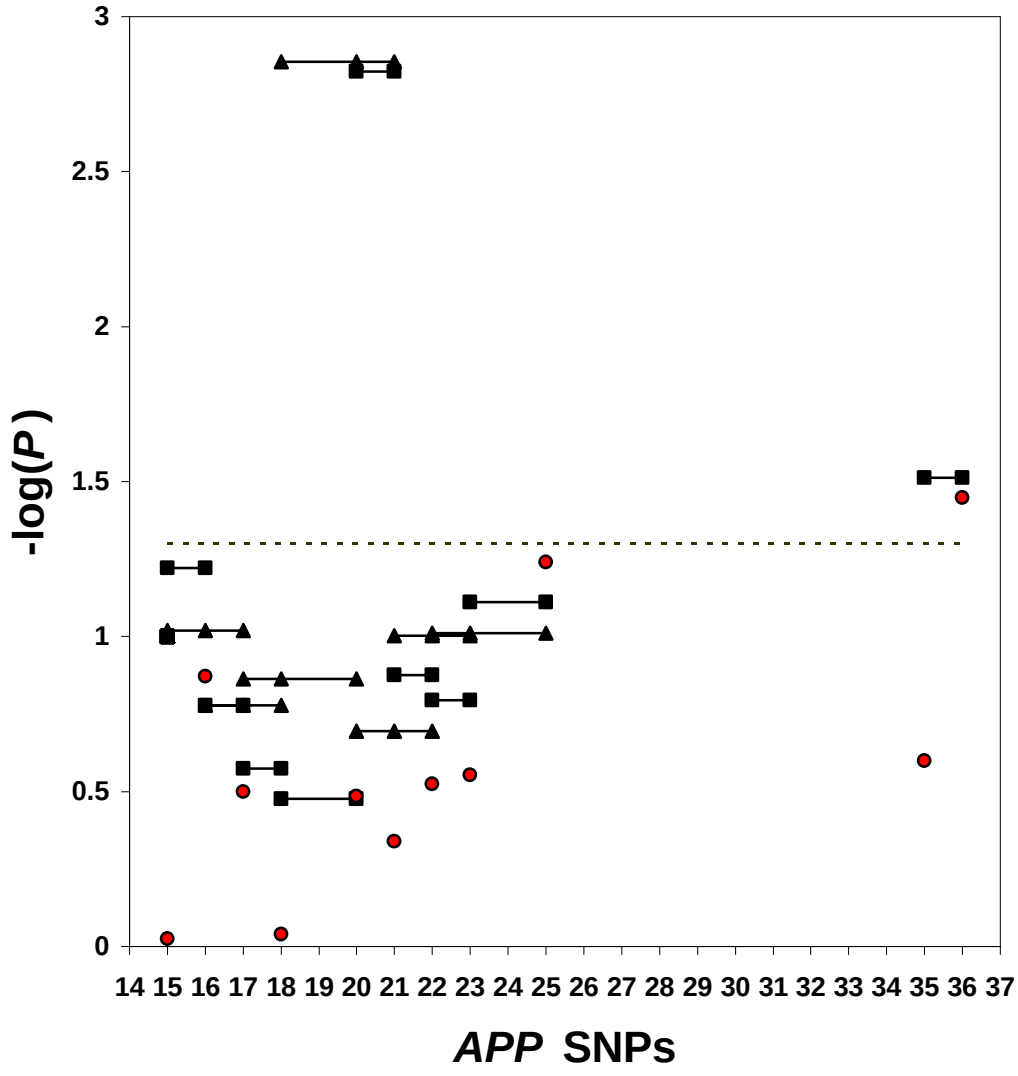

Fig. 38: Sliding window analysis of 2- and 3-marker haplotypes for *APP* – Swedish study cohort

Tab. 71: Results from sliding window analysis of 2-marker haplotypes for *APP* – pooled study cohort

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	15-16	1.1	TG	0.715	0.0704
		1.2	TA	0.245	0.0601
2	16-17	2.1	GC	0.657	0.1669
		2.2	AT	0.240	0.4411
		2.3	GT	0.068	0.9849
3	17-18	3.1	CA	0.689	0.3445
		3.2	TA	0.270	0.2662
4	18-20	4.1	AC	0.616	0.3335
		4.2	AT	0.344	0.3410

5	20-21	5.1	CC	0.646	0.6930
		5.2	TA	0.291	0.2250
		5.3	TC	0.053	0.6088
6	21-22	6.1	CG	0.539	0.1328
		6.2	AG	0.255	0.4505
		6.3	CT	0.164	0.2579
7	22-23	7.1	GC	0.741	0.2745
		7.2	TT	0.197	0.1602
		7.3	GT	0.054	0.8640
8	23-25	8.1	CC	0.495	0.5337
		8.2	CT	0.254	0.0775
		8.3	TC	0.245	0.2760
9	35-36	9.1	TG	0.688	0.3054
		9.2	CC	0.260	**0.0308**

Tab. 72: Results from sliding window analysis of 3-marker haplotypes for *APP* – pooled study cohort

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	15-17	1.1	TGC	0.647	0.0956
		1.2	TAT	0.211	0.2280
		1.3	TGT	0.066	0.9348
2	16-18	2.1	GCA	0.655	0.1665
		2.2	ATA	0.203	0.3393
		2.3	GTA	0.067	0.8984
3	17-20	3.1	CAC	0.357	0.6025
		3.2	CAT	0.331	0.1368
		3.3	TAC	0.259	0.5614
4	18-21	4.1	ACC	0.608	0.6873
		4.2	ATA	0.290	0.2337
		4.3	ATC	0.053	0.6097
5	20-22	5.1	CCG	0.485	0.2094
		5.2	TAG	0.248	0.2018
		5.3	CCT	0.161	0.2426
		5.4	TCG	0.051	0.6475
6	21-23	6.1	CGC	0.488	0.0993
		6.2	AGC	0.253	0.4442
		6.3	CTT	0.156	0.1627
		6.4	CGT	0.051	0.8619
7	22-25	7.1	GCC	0.489	0.6239
		7.2	GCT	0.252	0.0974
		7.3	TTC	0.195	0.1733

7.4.2 Results from Sliding Window Analyses for *BACE1*

Tab. 73: Results from sliding window analysis of 2-marker haplotypes for *BACE1*– complete Munich cohort

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-2	1.1	AT	0.584	0.8009
		1.2	TT	0.367	0.9540
2	2-3	2.1	TC	0.887	0.4526
		2.2	TG	0.064	0.5574
3	3-4	3.1	CG	0.593	0.8745
		3.2	CC	0.340	0.6887
		3.3	GC	0.063	0.6378
4	4-5	4.1	GT	0.597	0.8757
		4.2	CT	0.316	0.4818
		4.3	CC	0.087	0.3744
5	5-6	5.1	TG	0.768	0.2605
		5.2	TC	0.145	0.5260
		5.3	CG	0.087	0.3719
6	6-7	6.1	GA	0.523	0.2240
		6.2	GG	0.332	0.4134
		6.3	CA	0.145	0.5280
7	7-8	7.1	AT	0.347	0.9845
		7.2	GT	0.331	0.3930
		7.3	AG	0.321	0.4003
8	8-9	8.1	TA	0.408	0.5081
		8.2	GG	0.322	0.3854
		8.3	TG	0.270	0.8565
9	9-10	9.1	GA	0.591	0.5751
		9.2	AA	0.313	0.5020
		9.3	AG	0.094	0.9601
10	10-11	10.1	AA	0.561	0.3955
		10.2	AC	0.344	0.3288
		10.3	GC	0.092	0.9093

Tab. 74: Results from sliding window analysis of 3-marker haplotypes for *BACE1*– complete Munich cohort

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-3	1.1	ATC	0.519	0.5868
		1.2	TTC	0.367	0.9413
		1.3	ATG	0.065	0.5718
2	2-4	2.1	TCG	0.545	0.9567
		2.2	TCC	0.340	0.6685
		2.3	TGC	0.063	0.6250
3	3-5	3.1	CGT	0.593	0.9028
		3.2	CCT	0.256	0.3677
		3.3	CCC	0.085	0.4146
		3.4	GCT	0.061	0.7349
4	4-6	4.1	GTG	0.452	0.7767
		4.2	CTG	0.316	0.4781
		4.3	GTC	0.145	0.5311

		4.4	CCG	0.087	0.3744
5	5-7	5.1	TGA	0.523	0.2303
		5.2	TGG	0.244	0.7752
		5.3	TCA	0.145	0.5280
		5.4	CGG	0.087	0.3673
6	6-8	6.1	GGT	0.331	0.3963
		6.2	GAG	0.322	0.3838
		6.3	GAT	0.201	0.6151
		6.4	CAT	0.145	0.5528
7	7-9	7.1	GTA	0.331	0.3725
		7.2	AGG	0.322	0.3936
		7.3	ATG	0.268	0.8479
		7.4	ATA	0.079	0.6942
8	8-10	8.1	GGA	0.322	0.3852
		8.2	TAA	0.314	0.4859
		8.3	TGA	0.269	0.7899
		8.4	TAG	0.094	0.9626
9	9-11	9.1	GAA	0.523	0.4321
		9.2	AAC	0.276	0.4237
		9.3	AGC	0.092	0.9771
		9.4	GAC	0.068	0.7540

7.4.3 Results from Sliding Window Analyses for *ADAM10*

Tab. 75: Results from sliding window analysis of 2-marker haplotypes for *ADAM10*– complete Munich cohort

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-2	1.1	GA	0.671	0.9598
		1.2	GG	0.217	0.9252
		1.3	TA	0.112	0.9619
2	2-3	2.1	AA	0.668	0.6879
		2.2	GA	0.217	0.9331
		2.3	AG	0.115	0.6283
3	3-4	3.1	AG	0.880	0.5749
		3.2	GT	0.117	0.5225
4	4-5	4.1	GT	0.880	0.6449
		4.2	TC	0.117	0.5199
5	5-6	5.1	TA	0.628	0.8865
		5.2	TG	0.255	0.8515
		5.3	CG	0.117	0.6389
6	6-7	6.1	AC	0.624	0.8413
		6.2	GT	0.330	0.9320
7	7-8	7.1	CG	0.666	0.9331
		7.2	TG	0.223	0.7953
		7.3	TA	0.109	0.6156
8	8-9	8.1	GA	0.881	0.6184
		8.2	AG	0.111	0.7723
9	9-10	9.1	AA	0.880	0.6438
		9.2	GT	0.119	0.6178
10	10-11	10.1	AG	0.883	0.7611
		10.2	TA	0.107	0.7442
11	11-12	11.1	GA	0.880	0.4470
		11.2	AC	0.106	0.6396
12	12-13	12.1	AA	0.877	0.5317

		12.2	CT	0.115	0.6582
13	13-14	13.1	AC	0.880	0.6729
		13.2	TG	0.117	0.5593
14	14-15	14.1	CC	0.771	0.9431
		14.2	GC	0.118	0.4901
		14.3	CT	0.111	0.5391
15	15-16	15.1	CA	0.759	0.7144
		15.2	CG	0.127	0.4032
		15.3	TG	0.114	0.7017
16	16-17	16.1	AC	0.755	0.7884
		16.2	GT	0.234	0.7218
17	17-18	17.1	CT	0.767	0.8129
		17.2	TT	0.124	0.8620
		17.3	TC	0.107	0.7307
18	18-19	18.1	TG	0.750	0.6723
		18.2	TC	0.142	0.6893
		18.3	CG	0.108	0.8881
19	19-20	19.1	GG	0.587	0.6888
		19.2	GA	0.272	0.8908
		19.3	CG	0.141	0.6960
20	20-21	20.1	GC	0.580	0.4101
		20.2	GA	0.148	0.2893
		20.3	AC	0.144	0.6346
		20.4	AA	0.128	0.6836
21	21-22	21.1	CC	0.723	0.5686
		21.2	AC	0.169	0.7897
		21.3	AA	0.108	0.6173
22	22-23	22.1	CG	0.889	0.7047
		22.2	AA	0.106	0.6242
23	23-24	23.1	GG	0.886	0.7083
		23.2	AA	0.108	0.4882
24	24-25	24.1	GG	0.695	0.9938
		24.2	GA	0.195	0.5265
		24.3	AA	0.102	0.2988
25	25-26	25.1	GA	0.683	0.9433
		25.2	AG	0.260	0.4623
26	26-27	26.1	AT	0.712	0.7762
		26.2	GC	0.267	0.4378

Tab. 76: Results from sliding window analysis of 3-marker haplotypes for *ADAM10*– complete Munich cohort

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-3	1.1	GAA	0.665	0.6759
		1.2	GGA	0.217	0.9484
		1.3	TAG	0.113	0.6963
2	2-4	2.1	AAG	0.664	0.6454
		2.2	GAG	0.217	0.9316
		2.3	AGT	0.117	0.5225
3	3-5	3.1	AGT	0.880	0.5911
		3.2	GTC	0.117	0.5377
4	4-6	4.1	GTA	0.627	0.8617
		4.2	GTG	0.252	0.8801
		4.3	TCG	0.117	0.5199
5	5-7	5.1	TAC	0.624	0.8203
		5.2	TGT	0.215	0.7184
		5.3	CGT	0.116	0.7062

6	6-8	6.1	ACG	0.624	0.8418
		6.2	GTG	0.221	0.6627
		6.3	GTA	0.109	0.6408
7	7-9	7.1	CGA	0.665	0.9411
		7.2	TGA	0.217	0.7880
		7.3	TAG	0.110	0.7373
8	8-10	8.1	GAA	0.880	0.6438
		8.2	AGT	0.111	0.7724
9	9-11	9.1	AAG	0.880	0.6439
		9.2	GTA	0.108	0.6473
10	10-12	10.1	AGA	0.879	0.5728
		10.2	TAC	0.107	0.6479
11	11-13	11.1	GAA	0.880	0.3830
		11.2	ACT	0.108	0.6390
12	12-14	12.1	AAC	0.875	0.6713
		12.2	CTG	0.117	0.6426
13	13-15	13.1	ACC	0.769	0.8935
		13.2	TGC	0.117	0.5620
		13.3	ACT	0.111	0.5391
14	14-16	14.1	CCA	0.756	0.7673
		14.2	CTG	0.119	0.7657
		14.3	GCG	0.118	0.5575
15	15-17	15.1	CAC	0.755	0.7959
		15.2	CGT	0.119	0.4240
		15.3	TGT	0.114	0.7193
16	16-18	16.1	ACT	0.753	0.9050
		16.2	GTT	0.126	0.8957
		16.3	GTC	0.108	0.7217
17	17-19	17.1	CTG	0.625	0.6036
		17.2	CTC	0.141	0.6991
		17.3	TTG	0.124	0.8431
		17.4	TCG	0.107	0.7112
18	18-20	18.1	TGG	0.579	0.5737
		18.2	TGA	0.171	0.8152
		18.3	TCG	0.141	0.6962
		18.4	CGA	0.106	0.7648
19	19-21	19.1	GGC	0.573	0.6272
		19.2	GAC	0.143	0.7021
		19.3	GAA	0.137	0.9936
		19.4	CGA	0.131	0.4775
20	20-22	20.1	GCC	0.577	0.4978
		20.2	ACC	0.146	0.8156
		20.3	GAC	0.144	0.6183
		20.4	AAA	0.105	0.6859
21	21-23	21.1	CCG	0.720	0.6872
		21.2	ACG	0.170	0.7978
		21.3	AAA	0.106	0.7051
22	22-24	22.1	CGG	0.886	0.6502
		22.2	AAA	0.109	0.5044
23	23-25	23.1	GGG	0.693	0.9737
		23.2	GGA	0.192	0.7331
		23.3	AAA	0.100	0.3434
24	24-26	24.1	GGA	0.683	0.8978
		24.2	GAG	0.159	0.9057
		24.3	AAG	0.103	0.3175
25	25-27	25.1	GAT	0.679	0.9028
		25.2	AGC	0.258	0.3560

7.4.4 Results from Sliding Window Analyses for *ABCA1*

Tab. 77: Results from sliding window analysis of 2-marker haplotypes for *ABCA1* – complete Munich cohort

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-2	1.1	TG	0.512	0.1751
		1.2	CG	0.365	**0.0273**
		1.3	TC	0.081	0.3463
2	2-3	2.1	GG	0.755	0.2023
		2.2	GA	0.122	0.6240
		2.3	CG	0.121	0.2897
3	3-4	3.1	GT	0.751	0.8708
		3.2	AT	0.125	0.6005
		3.3	GC	0.124	0.4606
4	4-5	4.1	TG	0.734	0.7056
		4.2	TA	0.142	0.7111
		4.3	CA	0.122	0.2707
5	5-6	5.1	GG	0.651	0.8671
		5.2	AG	0.265	0.5741
		5.3	GA	0.084	0.5447
6	6-7	6.1	GT	0.863	0.3654
		6.2	AT	0.084	0.5328
		6.3	GG	0.052	0.5367
7	7-8	7.1	TC	0.912	0.6536
8	8-9	8.1	CA	0.879	0.2430
		8.2	CG	0.073	0.6303
9	9-10	9.1	AG	0.857	0.1928
		9.2	GG	0.115	0.1825
10	10-11	10.1	GA	0.908	0.3810
		10.2	GC	0.065	0.3628
11	11-12	11.1	AC	0.828	0.3477
		11.2	AG	0.104	0.6243
		11.3	CC	0.067	0.4146
12	12-13	12.1	CA	0.850	0.4553
		12.2	GA	0.104	0.6228
13	13-14	13.1	AA	0.882	0.9377
		13.2	AG	0.072	0.7253
14	14-15	14.1	AG	0.783	0.8274
		14.2	AT	0.145	0.9976
		14.3	GG	0.072	0.7253
15	15-16	15.1	GG	0.693	0.7130
		15.2	GA	0.162	0.6235
		15.3	TA	0.145	0.9743
16	16-17	16.1	GG	0.685	0.7953
		16.2	AA	0.269	0.4722
17	17-18	17.1	GG	0.631	0.1352
		17.2	AG	0.219	0.6691
		17.3	GA	0.090	0.1024
		17.4	AA	0.060	0.7459
18	18-19	18.1	GG	0.542	0.4005
		18.2	GA	0.308	0.7151
		18.3	AA	0.147	0.1414
19	19-20	19.1	GC	0.544	0.5085
		19.2	AC	0.434	0.2750
20	20-21	20.1	CG	0.653	0.3603

		20.2	CA	0.326	0.6459
21	21-22	21.1	GC	0.634	0.5915
		21.2	AC	0.237	0.4736
		21.3	AG	0.090	0.7522
22	22-23	22.1	CC	0.614	0.9967
		22.2	CT	0.257	0.9043
		22.3	GT	0.127	0.8440
23	23-24	23.1	CA	0.434	0.7060
		23.2	TA	0.347	0.9120
		23.3	CG	0.182	0.6934
24	24-25	24.1	AG	0.609	0.2731
		24.2	AA	0.174	0.4318
		24.3	GG	0.173	0.7988
25	25-26	25.1	GT	0.444	0.4144
		25.2	GC	0.338	0.8862
		25.3	AT	0.214	0.2287
26	26-27	26.1	TG	0.361	0.4327
		26.2	CA	0.312	0.7880
		26.3	TA	0.296	0.4860
27	27-28	27.1	AA	0.587	0.5455
		27.2	GG	0.285	0.1584
		27.3	GA	0.108	0.1496
28	28-29	28.1	AA	0.402	0.1037
		28.2	GA	0.304	0.1272
		28.3	AG	0.295	0.8329
29	29-30	29.1	AG	0.568	0.6663
		29.2	GA	0.290	0.9912
		29.3	AA	0.139	0.5064

Tab. 78: Results from sliding window analysis of 3-marker haplotypes for *ABCA1* – complete Munich cohort

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-3	1.1	TGG	0.423	0.3211
		1.2	CGG	0.332	**0.0254**
		1.3	TGA	0.088	0.5086
		1.4	TCG	0.082	0.3400
2	2-4	2.1	GGT	0.632	0.6520
		2.2	GAT	0.123	0.6773
		2.3	GGC	0.122	0.3531
		2.4	CGT	0.120	0.3433
3	3-5	3.1	GTG	0.734	0.6831
		3.2	ATA	0.130	0.6874
		3.3	GCA	0.124	0.2020
4	4-6	4.1	TGG	0.649	0.9977
		4.2	TAG	0.142	0.7168
		4.3	CAG	0.122	0.2712
		4.4	TGA	0.084	0.5448
5	5-7	5.1	GGT	0.627	0.9645
		5.2	AGT	0.236	0.4500
		5.3	GAT	0.084	0.5462
6	6-8	6.1	GTC	0.842	0.5895
		6.2	ATC	0.070	0.1932
7	7-9	7.1	TCA	0.843	0.4265
		7.2	TCG	0.069	0.5012
8	8-10	8.1	CAG	0.854	0.2345
		8.2	CGG	0.070	0.6033
9	9-11	9.1	AGA	0.857	0.1978
		9.2	GGC	0.065	0.4070

10	10-12	10.1	GAC	0.829	0.4286
		10.2	GAG	0.079	0.8359
		10.3	GCC	0.065	0.3702
11	11-13	11.1	ACA	0.824	0.3048
		11.2	AGA	0.104	0.6237
12	12-14	12.1	CAA	0.777	0.6653
		12.2	GAA	0.104	0.6146
		12.3	CAG	0.072	0.7277
13	13-15	13.1	AAG	0.783	0.8274
		13.2	AAT	0.099	0.7177
		13.3	AGG	0.072	0.7253
14	14-16	14.1	AGG	0.621	0.8523
		14.2	AGA	0.162	0.6067
		14.3	ATA	0.145	0.9743
		14.4	GGG	0.072	0.7336
15	15-17	15.1	GGG	0.685	0.7973
		15.2	TAA	0.137	0.7951
		15.3	GAA	0.131	0.5031
16	16-18	16.1	GGG	0.603	0.1740
		16.2	AAG	0.211	0.5380
		16.3	GGA	0.082	**0.0474**
		16.4	AAA	0.058	0.7723
17	17-19	17.1	GGG	0.441	0.4410
		17.2	GGA	0.191	0.3006
		17.3	AGA	0.118	0.4370
		17.4	AGG	0.101	0.8724
		17.5	GAA	0.088	0.0838
		17.6	AAA	0.060	0.8917
18	18-20	18.1	GGC	0.542	0.4303
		18.2	GAC	0.289	0.9127
		18.3	AAC	0.145	0.1672
19	19-21	19.1	GCG	0.494	0.9525
		19.2	ACA	0.276	0.7739
		19.3	ACG	0.157	0.3058
		19.4	GCA	0.051	0.2371
20	20-22	20.1	CGC	0.634	0.5134
		20.2	CAC	0.237	0.3746
		20.3	CAG	0.091	0.5640
21	21-23	21.1	GCC	0.611	0.9463
		21.2	ACT	0.236	0.4317
		21.3	AGT	0.091	0.6922
22	22-24	22.1	CCA	0.432	0.6973
		22.2	CTA	0.247	0.8018
		22.3	CCG	0.182	0.6536
		22.4	GTA	0.101	0.7910
23	23-25	23.1	CAG	0.349	0.2427
		23.2	TAG	0.258	0.9598
		23.3	CGG	0.152	0.9527
		23.4	TAA	0.089	0.8116
		23.5	CAA	0.085	0.1877
24	24-26	24.1	AGT	0.350	0.4928
		24.2	AGC	0.258	0.5830
		24.3	AAT	0.171	0.3570
		24.4	GGT	0.094	0.7858
		24.5	GGC	0.080	0.5234
25	25-27	25.1	GCA	0.309	0.7552
		25.2	GTA	0.262	0.3723
		25.3	GTG	0.182	0.9980
		25.4	ATG	0.179	0.3017
26	26-28	26.1	CAA	0.305	0.8654
		26.2	TAA	0.282	0.3790
		26.3	TGG	0.262	0.0986

		26.4	TGA	0.101	0.2591
27	27-29	27.1	AAA	0.302	0.2797
		27.2	AAG	0.285	0.6784
		27.3	GGA	0.285	0.1598
		27.4	GAA	0.100	0.2957
28	28-30	28.1	AAG	0.335	0.3922
		28.2	AGA	0.290	0.9910
		28.3	GAG	0.234	0.1487
		28.4	GAA	0.071	0.6555
		28.5	AAA	0.068	0.1800

7.4.5 Results from Sliding Window Analyses for *LIPC*

Tab. 79: Results from sliding window analysis of 2-marker haplotypes for *LIPC* – complete Munich cohort

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-2	1.1	AC	0.52	0.9982
		1.2	CC	0.266	0.1649
		1.3	CT	0.197	0.1471
2	2-3	2.1	CT	0.45	0.5998
		2.2	CC	0.335	0.4996
		2.3	TC	0.215	0.1581
3	3-4	3.1	TG	0.44	0.6028
		3.2	CA	0.282	0.8651
		3.3	CG	0.269	0.8656
4	4-5	4.1	GG	0.529	0.7672
		4.2	AG	0.19	0.0873
		4.3	GC	0.183	0.3569
		4.4	AC	0.098	0.1205
5	5-6	5.1	GA	0.553	0.7796
		5.2	CA	0.279	0.0791
		5.3	GG	0.165	0.0675
6	6-7	6.1	AT	0.53	0.4295
		6.2	AC	0.302	0.5984
		6.3	GT	0.143	0.1083
7	7-8	7.1	TT	0.587	0.6074
		7.2	CT	0.327	0.7151
		7.3	TA	0.086	0.7710
8	8-9	8.1	TC	0.802	0.7458
		8.2	TT	0.112	0.5078
		8.3	AC	0.086	0.7769
9	9-10	9.1	CC	0.843	0.7638
		9.2	TG	0.153	0.9512
10	10-11	10.1	CC	0.562	0.5151
		10.2	CG	0.285	0.4891
		10.3	GG	0.151	0.8850
11	11-12	11.1	GG	0.449	0.4425
		11.2	CC	0.415	0.6364
		11.3	CG	0.127	0.7215
12	12-13	12.1	CT	0.419	0.5259
		12.2	GC	0.356	0.3681
		12.3	GT	0.22	0.7162
13	13-14	13.1	TC	0.49	0.6809
		13.2	CC	0.319	0.3371

		13.3	TG	0.15	0.0669
14	14-15	14.1	CT	0.627	0.8983
		14.2	GC	0.195	0.1421
		14.3	CC	0.178	0.1740
15	15-16	15.1	TG	0.552	0.2027
		15.2	CG	0.259	0.4804
		15.3	CA	0.115	0.4597
		15.4	TA	0.074	**0.0319**
16	16-17	16.1	GG	0.791	0.5020
		16.2	AG	0.188	0.3998
17	17-18	17.1	GC	0.976	0.6069
18	18-19	18.1	CG	0.945	0.7268
19	19-20	19.1	GG	0.666	0.7640
		19.2	GT	0.298	0.5619
20	20-21	20.1	GC	0.56	0.9986
		20.2	TC	0.319	0.7496
		20.3	GT	0.122	0.6509
21	21-22	21.1	CG	0.866	0.9775
		21.2	TG	0.121	0.6618
22	22-23	22.1	GA	0.988	0.1688
23	23-24	23.1	AC	0.865	0.9528
		23.2	AA	0.123	0.7620
24	24-25	24.1	CT	0.511	0.4385
		24.2	CC	0.365	0.5624
		24.3	AT	0.123	0.7418

Tab. 80: Results from sliding window analysis of 3-marker haplotypes for *LIPC* – complete Munich cohort

Window	Marker	Haplotyp ID	Sequence	Frequency	p-values
1	1-3	1.1	ACT	0.439	0.8344
		1.2	CCC	0.255	0.1705
		1.3	CTC	0.195	0.1230
		1.4	ACC	0.079	0.5030
2	2-4	2.1	CTG	0.439	0.6020
		2.2	CCG	0.259	0.9291
		2.3	TCA	0.202	0.1693
		2.4	CCA	0.078	0.0959
3	3-5	3.1	TGG	0.437	0.5744
		3.2	CAG	0.192	0.1211
		3.3	CGC	0.189	0.4438
		3.4	CAC	0.089	0.0542
		3.5	CGG	0.081	0.1117
4	4-6	4.1	GGA	0.510	0.9832
		4.2	GCA	0.190	0.4878
		4.3	AGG	0.155	0.0804
		4.4	ACA	0.089	0.0675
5	5-7	5.1	GAT	0.315	0.6067
		5.2	GAC	0.238	0.8349
		5.3	CAT	0.216	0.1146
		5.4	GGT	0.142	0.1045
		5.5	CAC	0.063	0.5580
6	6-8	6.1	ATT	0.477	0.8693
		6.2	ACT	0.303	0.6089
		6.3	GTT	0.110	0.2846
		6.4	ATA	0.053	0.1582
7	7-9	7.1	TTC	0.476	0.9865

		7.2	CTC	0.323	0.7710
		7.3	TTT	0.110	0.4320
		7.4	TAC	0.086	0.7769
8	8-10	8.1	TCC	0.757	0.6634
		8.2	TTG	0.153	0.9506
		8.3	ACC	0.086	0.7813
9	9-11	9.1	CCC	0.560	0.4931
		9.2	CCG	0.284	0.5112
		9.3	TGG	0.149	0.9836
10	10-12	10.1	CCC	0.412	0.7195
		10.2	CGG	0.301	0.5035
		10.3	GGG	0.148	0.7597
		10.4	CCG	0.127	0.6541
11	11-13	11.1	CCT	0.410	0.5965
		11.2	GGC	0.246	0.2460
		11.3	GGT	0.203	0.7909
		11.4	CGC	0.110	0.8401
12	12-14	12.1	GCC	0.314	0.3882
		12.2	CTC	0.306	0.7849
		12.3	GTC	0.183	0.8214
		12.4	CTG	0.113	0.1700
13	13-15	13.1	TCT	0.331	0.7749
		13.2	CCT	0.296	0.6811
		13.3	TCC	0.156	0.3927
		13.4	TGC	0.152	0.0805
14	14-16	14.1	CTG	0.555	0.0868
		14.2	GCG	0.194	0.2401
		14.3	CCA	0.113	0.1606
		14.4	CTA	0.071	**0.0065**
		14.5	CCG	0.062	0.8279
15	15-17	15.1	TGG	0.545	0.2335
		15.2	CGG	0.246	0.4484
		15.3	CAG	0.115	0.4501
		15.4	TAG	0.074	**0.0306**
16	16-18	16.1	GGC	0.791	0.5029
		16.2	AGC	0.186	0.3676
17	17-19	17.1	GCG	0.944	0.8279
18	18-20	18.1	CGG	0.655	0.8132
		18.2	CGT	0.290	0.6745
19	19-21	19.1	GGC	0.545	0.9588
		19.2	GTC	0.298	0.5906
		19.3	GGT	0.122	0.6512
20	20-22	20.1	GCG	0.560	0.9984
		20.2	TCG	0.306	0.9933
		20.3	GTG	0.122	0.6515
21	21-23	21.1	CGA	0.866	0.9775
		21.2	TGA	0.121	0.6618
22	22-24	22.1	GAC	0.864	0.8774
		22.2	GAA	0.123	0.7621
23	23-25	23.1	ACT	0.500	0.5952
		23.2	ACC	0.365	0.5647
		23.3	AAT	0.123	0.7432

7.5 Applied Primers

7.5.1 Applied Primers for Genotyping

7.5.1.1 Applied Primers for Genotyping of SNPs in *APP*

Tab. 81: Applied primers for genotyping of SNPs in *APP* in initial case-control cohort from Munich

SNP ID	1st Primer	2nd Primer	xt Primer	AMP Len
rs2040124	ACGTTGGATGTGGCAGTTGTTCGTTCCATC	ACGTTGGATGTGCCTACCTGCCAACTAAAC	TCTTCCTTATCCCCAAAAT	106
rs2829955	ACGTTGGATGCAGTCATGCTTTTTAACAG	ACGTTGGATGTGGTGCATTTTCGGCTGAAG	GAAGCCAGTGTTTTGTT	93
rs6516704	ACGTTGGATGAACAGGACACAGATGTTTTC	ACGTTGGATGTCTTGGACTTCAATTCTGGG	CTCTAGATGACTATTGTCCTTTTTTT	99
rs6516708	ACGTTGGATGAACATCCGCTTCCTGGGTTC	ACGTTGGATGTGGCACACGCCTGTTAAGTC	CGCCTGTTAAGTCCCAGCTACTC	96
rs11549660	ACGTTGGATGAGACCCAAAGATACGTGGAC	ACGTTGGATGCGTTTGTTTCTTCGTGCCTG	CATTAGGCATTGAGACTTCAAGCTTTT	108
rs373521	ACGTTGGATGTATTGTGTCTGTGCAGAGG	ACGTTGGATGCTTCCTCATGGCTAAGCTAC	CCAGCCCTACCACGAC	118
rs2070653	ACGTTGGATGAAAGGTAAGAGTCTGCACTG	ACGTTGGATGTTGTCTTAAATAGTCTGGC	CCCTAAATAGTCTGGCCTCTCAA	99
rs2829975	ACGTTGGATGGGATTTGCATTCTTAACTCCC	ACGTTGGATGCTTGCCAATGAAAACCATGC	GCTTCAAAAAGCTAAATGTCTT	100
rs1783016	ACGTTGGATGACCTCTGGGATTTTTGAGTC	ACGTTGGATGTTGGCCTGGTAGAGAGTTTG	TTGTACAAGGCAGCAC	116
rs2829983	ACGTTGGATGGCAGTAATTACACATGATAC	ACGTTGGATGCAAGAATCAGCTTTATGGAAC	AGCTTTATGGAACATCATTGACTTATC	113
rs216781	ACGTTGGATGGAACATATTACGTATGTGCTC	ACGTTGGATGCTAATGGGTGGATTGAGAGC	GATTGAGAGCATATTACATACA	113
rs2186301	ACGTTGGATGAAAGAATACACTTTCTAGG	ACGTTGGATGTCTGCCAGAATGGTAGAGAG	TGGTAGAGAGCGCAAT	104
rs216762	ACGTTGGATGCATTTGTTTGCCTTTCTGAAG	ACGTTGGATGTTTAATGCCAGGAGTTGAGC	AAGTCTCCTGCAAAATTTATC	103
rs762479	ACGTTGGATGCACCTGCTTTAACAGTGTGG	ACGTTGGATGTCGGCCTCGCAAAGTGTTG	CCCAGCCCAGATTTTTTT	120
rs9978555	ACGTTGGATGCTTCAGTCCCATGTGTGAAC	ACGTTGGATGGAAGCTGCAATCATCCAATC	CTGCAATCATCCAATCAGAGCCAG	113
rs9941877	ACGTTGGATGCTGTCTCTATAGATTTGCCC	ACGTTGGATGGAAGTCGGACACAAAAGACC	CCATGTATTATATGATCCCACTTA	100
rs2830012	ACGTTGGATGGAAAGATGACCTGATGCATGT	ACGTTGGATGAAACAGCGCTAGTTAGTGCC	GATCTCAAACACGGTAAAT	113
rs2830019	ACGTTGGATGGCTTCCTATCTGAACTTTTC	ACGTTGGATGGCCAGAATATCATTTTCCCC	CTCTTTAAACATACTTTTTATGATGCC	98
rs768039	ACGTTGGATGATTACAAGCACCCGCCACAC	ACGTTGGATGCTAACACAGAGAAACCCCG	CCCGTGTCTACTAAAAAATC	92
rs3787644	ACGTTGGATGGAGGAAAAGTGTGACTCTAC	ACGTTGGATGGATTGAACCCAGTGGTCCAT	CGTTGTCCATGGGGGTTGCTGAATT	114
rs2070655	ACGTTGGATGGGTTCCTAATATATTGGGTC	ACGTTGGATGAACAGTGAGTGGTAGAGTAG	TGGTAGAGTAGATGTGTTTCATT	106
rs3991	ACGTTGGATGCAGACAGTAAGTGCCAAACC	ACGTTGGATGATCTAGTCGTCAGCAGATTG	CGTCAGCAGATTGACTAAGG	96
rs2830035	ACGTTGGATGGGAGTAAGAGTCTGAAATGG	ACGTTGGATGGTGTATATGAAAATGACAGC	TGACAGCTACAGATAAATGTTTTCTTA	97
rs743532	ACGTTGGATGTCTTGAACCTTGCCCTTGAC	ACGTTGGATGGTTACTGGGTTGTTTTGTTG	TGCCCTTGATTCTTTGA	110
rs1041420	ACGTTGGATGGAGAGGGAAACTGAAGATGG	ACGTTGGATGCCATCCTATTACCCCAAACG	TCTTCCAGGTCAGCAATCCAAAGTCAC	96
rs2830046	ACGTTGGATGTTATCACCTGGGTTTTGCTG	ACGTTGGATGGGGATACTCTATTTCAGGGC	ATTATGTCTAGAGCTCATAACTCTTA	118

rs8130594	ACGTTGGATGCTTCCCATTCTCTCATGACC	ACGTTGGATGCATCCATCCTATTAAGTCTG	GCTGTATTCAAAGGATGAACTGATGA	112
rs2830053	ACGTTGGATGCTCAGGATTATGATGTGCTAC	ACGTTGGATGTGTCATAACTGAGGGTTTCC	CTGAGGGTTTCCTAAGTA	94
rs2246115	ACGTTGGATGATGTGTGTATGTTGACTACC	ACGTTGGATGATGAAGACAACATGAGACAG	AAGACAACATGAGACAGTTATAG	116
rs2830069	ACGTTGGATGCTGCTGTTGTTCATAGCATGT	ACGTTGGATGTAGCACATCTGAAGAGGCAC	CTGAAGAGGCACTTCTATTCT	100
rs462278	ACGTTGGATGAGCTGTCACTAGATAAAAGG	ACGTTGGATGCCATGCCAAAGTACTTTTATC	ACTTTTATCTTAAATTGCTTATTTTT	116
rs2830082	ACGTTGGATGCGGAGGTTTGCTCCTTCATT	ACGTTGGATGTGTTCTTAGCGTCTGTGGTC	GAAGAGCTCTCTGGTCTTGTTT	110
rs1788283	ACGTTGGATGTAGCCTTGGCAGCAGACTGA	ACGTTGGATGCTTAAGTGATGCTGAGTGAC	TTTTGTTTTTTTTCACTTTTTCTTTAC	112
rs2830092	ACGTTGGATGTAAGAGCCACCTGAAACCAC	ACGTTGGATGTTGGACCTTAGATTCGGGAG	ATTCGGGAGAAAATCACTGT	80
rs6516727	ACGTTGGATGGACTTTAGAGACTCACGAGG	ACGTTGGATGCACTTAAGTAGGGTACCTTG	CACTTAAGTAGGGTACCTTGTACCTAA	110
rs2830099	ACGTTGGATGGGCTAGCAGATAAGAGAAGG	ACGTTGGATGCGTTCTGAACCAAAGCTAAG	TCTCCGTGAGCGATTA	98
rs465984	ACGTTGGATGTGGTGCAGGGTATTCTCAAC	ACGTTGGATGTCCTGCATTCAGCCCCTTAC	CCTGCATTCAGCCCCTTACCTTTGA	100
rs1235885	ACGTTGGATGATACTTTCCCCGTGGACAAG	ACGTTGGATGTCAGATGGAATAGGCAGCAG	GGAATAGGCAGCAGTGGGCAA	120
rs456565	ACGTTGGATGCAGAAGACAGGAAGATGAGG	ACGTTGGATGGCCTGGACTTCACTATTCAT	TGACAACCATTTAACCAGT	120

Tab. 82: Applied primers for genotyping of SNPs in *APP* in replication studies

SNP ID	1st Primer	2nd Primer	xt Primer	AMP_Len
rs9978555	ACGTTGGATGTGTGAACAGGTGTAGGAAC	ACGTTGGATGGAAGCTGCAATCATCCAATC	CTAATCCAATCAGAGCCAG	100
rs9941877	ACGTTGGATGCTGTCTCTATAGATTTGCCC	ACGTTGGATGGAAGTCGGACACAAAAGACC	CATGTATTATATGATCCCACTTA	100
rs2830012	ACGTTGGATGTAGTGCCCATGATTTGATTG	ACGTTGGATGGAAAGATGACCTGATGCATGT	TTTGTCTGATGCATGTCATCAGTT	100
rs2830019	ACGTTGGATGGCCAGAATATCATTTTCCCC	ACGTTGGATGGCTTCCTATCTGAACTTTTC	ATCTGAACTTTTCTTTAAAATATCT	98
rs768039	ACGTTGGATGTTTCTTCTCCCACACCAAGC	ACGTTGGATGGTTCCAGGATCTGCTTTATG	ACTCTACATCCTGGCT	113
rs3787644	ACGTTGGATGTCTGATTGAACCCAGTGGTC	ACGTTGGATGGAGGAAAAGTGTGACTCTAC	TCTAACATTTCATTTGCTTTAC	113
rs2070655	ACGTTGGATGTATATTGGGTCTGCATGTTG	ACGTTGGATGGAAAACAGTGAGTGGTAGAG	GTAGAGTAGATGTGTTTCATT	106
rs3991	ACGTTGGATGCAGACAGTAAGTGCCAAACC	ACGTTGGATGATCTAGTCGTCAGCAGATTG	CGTCAGCAGATTGACTAAGG	96
rs2830035	ACGTTGGATGGTGTATATGAAAATGACAGC	ACGTTGGATGGGAGTAAGAGTCTGAAATGG	TGAAATGGTAAAATAAAAAATCAC	97
rs1041420	ACGTTGGATGGGAGAGGGAAACTGAAGATG	ACGTTGGATGCCCATCCTATTACCCCAAAC	CCCTTTCAGCAATCCAAAGTCAC	98
rs6516727	ACGTTGGATGTTTAGAGACTCACGAGGAGG	ACGTTGGATGCACTTAAGTAGGGTACCTTG	TAGGGTACCTTGTACCTAA	110
rs2830099	ACGTTGGATGGGCTAGCAGATAAGAGAAGG	ACGTTGGATGCGTTCTGAACCAAAGCTAAG	TCTCCGTGAGCGATTA	98

7.5.1.2 Applied Primers for Genotyping of SNPs in BACE1

Tab. 83: Applied primers for genotyping of SNPs in *BACE1* in initial case-control cohort from Munich

SNP ID	1st Primer	2nd Primer	xt Primer	AMP Len
rs644215	ACGTTGGATGCTAGCATCATGACTCTAGAC	ACGTTGGATGTTCCTCCTTTCTACCCTCAC	ACCCTCCTCTTTCAGGCCCC	109
rs12292027	ACGTTGGATGCCTAGCTATAGTAAATATGC	ACGTTGGATGATGAGAAGTCCTGAGGTATG	GAAGGCCTGAGGTATGAAGAGAAC	85
rs1047964	ACGTTGGATGGAATGATCTTGGGATGCTGG	ACGTTGGATGTCCATTTTTCCCAGGACAGG	CCGCCAGGAAATATGTCCCCC	102
rs638405	ACGTTGGATGGTCTCTGGTATACACCCATC	ACGTTGGATGTCCTGTCCATTGATCTCCAC	CTCCACCCGCACAATGAT	96
rs507805	ACGTTGGATGAAGTCTAAGCTGAACAGGCC	ACGTTGGATGTTCATGTCCTTGGACTATGC	GACTATGCAAAGCCACCT	115
rs609332	ACGTTGGATGGTCAAGGACCCCAGGATTTA	ACGTTGGATGATGATTCTTGTTTGGGCCAC	TTTTGGAGTAGTCTGTTACC	117
rs522843	ACGTTGGATGAGCTCAGGTCTGGAAATCAC	ACGTTGGATGCAGTTATAGTGACTCCAGGC	AGAACCTTGTGAGTCAGC	108
rs687740	ACGTTGGATGTTGCCCAGGCTGGAGTACAGG	ACGTTGGATGATCCAAGTTACTCAGGAGCC	TTGAGCCCAGGAGTTA	104
rs473210	ACGTTGGATGCCAGTTCTGCTGATTTCCTC	ACGTTGGATGTTGGGTACTATAGCCAGGTG	TAGCCAGGTGTTTCCCAAAATA	99
rs551662	ACGTTGGATGCATGGTGGTGACAGAGTAAG	ACGTTGGATGCAATCTTTGCAATCAATCTGG	GACAATCAATCTGGTTTTTTGT	100
rs525493	ACGTTGGATGTTGGCCTTCAGATATAAGGG	ACGTTGGATGGTCTCTTCCTGAAAGATTGC	TTACTGAAAGATTGCTTTCTTCATG	101

7.5.1.3 Applied Primers for Genotyping of SNPs in ADAM10

Tab. 84: Applied primers for genotyping of SNPs in *ADAM10* in initial case-control cohort from Munich

SNP ID	1st Primer	2nd Primer	xt Primer	AMP Len
rs12438487	ACGTTGGATGGCTACACCTTTTTCAGAAAC	ACGTTGGATGGATGCAGCATTTAGCAACTC	TTGAAAACATAAAGCAAGATAAT	117
rs1869135	ACGTTGGATGACTTCAGTGGTCGAACCATC	ACGTTGGATGCTGCACCGCATGAAAACATC	CCATCACCCTGCAACCT	105
rs7166076	ACGTTGGATGGAGTAAGAGGAGATCCTTTTG	ACGTTGGATGAGGTTTGAGGAAGGCACATC	GATCCTTTTGTAGTTCATAAGTGTG	104
rs7165035	ACGTTGGATGGTTTCTGCTGTCATATTTTGC	ACGTTGGATGGCATTTGGGAAACAGTGCTC	TGCTATAATTTTATGCATCTAAGTA	119
rs2305421	ACGTTGGATGCTTTGACCTACAGTTTGGCC	ACGTTGGATGGAGACACAATGCTACGTTAC	CCAGATAATTTCCAGCTAAATC	104
rs12594872	ACGTTGGATGAAAATAGGAAACCATGAGGG	ACGTTGGATGGGAAGTATGTAAATATCCTG	CATGAGGGAATAAATAAATGAATAC	97
rs1427282	ACGTTGGATGGCTGTTCTTGTCTCACTTAC	ACGTTGGATGTCTGAAGTATTTACAGGGAC	GTTCTTGTCTCACTTACTCCCACA	113

rs7163733	ACGTTGGATGCTTTGTGATTTTGGTAGAGC	ACGTTGGATGTGCAAGTGATAAGGTGAAAG	TGTGATTTTGGTAGAGCTTATTAAA	118
rs7174386	ACGTTGGATGCCCAGCATTTGAAGTTATAC	ACGTTGGATGACTTAACACAGGGTTCTCGG	GGTTAAGAATTCAATAAGCTG	102
rs9302203	ACGTTGGATGCAGGTTCCTCCTTCAGAATC	ACGTTGGATGACAGGTCTCTGAAAGGATGC	GGGATAATCTGTTCTAATACCAGAC	120
rs6494031	ACGTTGGATGGGTTGCACTATGTAAGAGAC	ACGTTGGATGCCTTAGACTTCGAGAAGGTC	TTGCACTATGTAAGAGACATTGGT	100
rs11071392	ACGTTGGATGGTTTCATTCCTCTGCATACG	ACGTTGGATGCAATGGTGCCAAAAACATCC	TTCCTCTGCATACGGATATCCAGTT	112
rs8026668	ACGTTGGATGTTAAACTGATGACAACAGTG	ACGTTGGATGGGGGACAAAGTTAAAGTGTA	GACAACAGTGATTGCATAA	102
rs11071393	ACGTTGGATGGAAGAGTTGTGATAGAGAAC	ACGTTGGATGCAGAGGAAATTCCTGCAAAC	ACTACATGTTGTTCAAAAGT	114
rs1427280	ACGTTGGATGCTTGTCATCTAGTAATGTTC	ACGTTGGATGAAGGAGGACACTAAGTCCAG	TCATCTAGTAATGTTCTTTGAAT	98
rs1427281	ACGTTGGATGGAACTACAGTGCATCCTATG	ACGTTGGATGCCTCATTTCTAGAAAAAGCC	CTATCCTATGATTTAGCATCTCA	101
rs4775086	ACGTTGGATGAACCATGGTTCACTTGACAG	ACGTTGGATGCGGAAGGAGTTCCCTTAAAC	CATGGTTCACTTGACAGATTTTTAG	116
rs2052805	ACGTTGGATGCACATCCTCAGGAACACATC	ACGTTGGATGGATGAAGATGAAGTGGCAAC	AACACATCACATCAGGA	90
rs2657125	ACGTTGGATGCAGAAATAGCCTGTTGAATAG	ACGTTGGATGGTTCCTTGTATATTCTGGGT	TATTTGCAAACTATTCATCTGA	102
rs347117	ACGTTGGATGCTTTGCACTAGTAAGTTTCC	ACGTTGGATGCTTGATTTGCTAGACCTTGTG	AATAGTCTATATTGAACCAACAA	98
rs4238331	ACGTTGGATGCTTTGTTAATTTAGGAGCATC	ACGTTGGATGCCCAAATCAATCAATATTGAG	ATTTAGGAGCATCAAATGTAT	95
rs12441313	ACGTTGGATGATGGGAGTCCTGGAATTCTC	ACGTTGGATGGAGGGGCTGATTATTTTGTG	TATGATCACAGCCCCGT	111
rs6494038	ACGTTGGATGAGCTTCAAAGTATGTGAAGA	ACGTTGGATGGCTTGTTCAGAAAGGAGTGG	AAAGTTGATAGAACAGAAAAAAG	114
rs12439231	ACGTTGGATGCATCTCAGCTACCATTGTCC	ACGTTGGATGTGCCCAAAAGACCTGGGTTG	AAAAAGGGGGACCTCTG	98
rs383902	ACGTTGGATGGTCAGCTTATTGATCTAAAG	ACGTTGGATGGACTAGGATTAATTTACAGAG	CTTATTGATCTAAAGTCTTCAATT	112
rs653765	ACGTTGGATGTTTCCTTCCCTTGCTCGTTC	ACGTTGGATGGTCACGTGGTGAGGAAGGAG	CTTCTCTCCCCCCCTCC	100
rs593742	ACGTTGGATGGAAGGCCAATGCTGGTAAAC	ACGTTGGATGACATCCAGCAGGCAGTTTAG	GGCCAATGCTGGTAAACATTTCAAC	95

7.5.1.4 Applied Primers for Genotyping of SNPs in *ABCA1*

Tab. 85: Applied primers for genotyping of SNPs in *ABCA1* in initial case-control cohort from Munich

SNP ID	1st Primer	2nd Primer	xt Primer	AMP Len
rs2515606	ACGTTGGATGAGGTAACGTAGAGTGTACCC	ACGTTGGATGAGACAGCGGTTTACCTTGAC	GAATGGGAAGCTGACATTTTT	105
rs1331924	ACGTTGGATGGATACAAACTGCTCTTGGAC	ACGTTGGATGACTTCTCAGGGTGTGGATTC	TGTGGATTCCTGCCCC	84
rs2066720	ACGTTGGATGCCAGCCCCATGCTTTTTCTT	ACGTTGGATGGATGGAGCAATTAGTCATCG	GCAATTAGTCATCGAGAAGA	116
rs2777799	ACGTTGGATGAGCCTCTATTCTTCTTCCCG	ACGTTGGATGAGACAGCGGTTTACCTTGAC	GCAGACTATAATATAGCATTAATGA	102
rs2230808	ACGTTGGATGCTGCAGATCGATTTCTCAAC	ACGTTGGATGAGACAGCGGTTTACCTTGAC	CAGCGGTTTACCTTGACATTATTT	102
rs2066716	ACGTTGGATGAAGAGGTCCATGATGGTCTG	ACGTTGGATGTCTTGCTGTCTTCCCTTTGC	CCCTTTGCAGAGACAC	114

rs2297401	ACGTTGGATGTGACTCAAGGTTTGCTGCTG	ACGTTGGATGAGACAGCGGTTTACCTTGAC	CCACCAAGCTGCTCCCAC	103
rs3818689	ACGTTGGATGCAAGAGCATACGTGTTACAG	ACGTTGGATGTTCATTATGCCTGCCAAAAG	GCCAAAAGTTACGTGC	98
rs4149313	ACGTTGGATGGTTTGGCGAGGAAAGTGATG	ACGTTGGATGAGACAGCGGTTTACCTTGAC	CAACAGCACTTACTTTCTGA	102
rs2066718	ACGTTGGATGTACTCACAGCGAAGATCTTG	ACGTTGGATGCATCTACTTCACGCTGTACC	CCTGTGTGTGGCATGGCAGGACTAC	109
rs2297398	ACGTTGGATGATCTCCCTGTGTCTTGGTAG	ACGTTGGATGAGACAGCGGTTTACCTTGAC	GACAGTCTACATGTAACATCATGC	94
rs2487062	ACGTTGGATGGTGTCAGGCAATGAAAAAGC	ACGTTGGATGAGACAGCGGTTTACCTTGAC	TGTGAGCCACTGCACC	115
rs914544	ACGTTGGATGCCTTTTAGGTCCACATGTTG	ACGTTGGATGAGACAGCGGTTTACCTTGAC	GAGGACAGAGATTACTCTGA	101
rs1175293	ACGTTGGATGGTGTGATACAGTCCCTGATG	ACGTTGGATGTCCATTCTTTAGGTCCCCAC	GTCCCCACTTAGAGGATGTTTC	107
rs2472445	ACGTTGGATGCACGGTGACTCATGCCTTTA	ACGTTGGATGAGACAGCGGTTTACCTTGAC	TGATCTGCCCACCTCG	113
rs2230806	ACGTTGGATGCTTATTGATGCAGGAAACTC	ACGTTGGATGAGACAGCGGTTTACCTTGAC	ACCTTTTTCCTTCCCTATTTAT	108
rs2230805	ACGTTGGATGCCTGGTGGACAATGAAACCT	ACGTTGGATGCAGCATCTTGTCCACAGTAG	GGAGAGAGAGGTTGTGATA	99
rs13301006	ACGTTGGATGATGACCTGATGTGATTTGGG	ACGTTGGATGTTTCCCTAACTCACTGCAAG	CTCCTTTTCTTCTCTGCAATCG	90
rs2000069	ACGTTGGATGCAGGTTTGATCACATGCACG	ACGTTGGATGAGACAGCGGTTTACCTTGAC	GGAAATGGTAAAAGCCACTTGTT	106
rs1999429	ACGTTGGATGACTGTAGAAGGAGAACTGCC	ACGTTGGATGTGTCCCAAGTCTACACAACC	GATTGGAATAAATCACGGCTAAA	112
rs2275542	ACGTTGGATGGCCACAGACAGTGATGTACT	ACGTTGGATGAGACAGCGGTTTACCTTGAC	CTCCAAGTCTGTAGTATTCTTC	107
rs3847305	ACGTTGGATGCCATAATGGCATATCCTGGC	ACGTTGGATGAGACAGCGGTTTACCTTGAC	CTCCCTCTAGAAACCAAA	101
rs2575875	ACGTTGGATGAGGCTGAACATAGCGTTTCT	ACGTTGGATGAGACAGCGGTTTACCTTGAC	AAATCATACATAAAGCCTGTGTGCTGC	103
rs3758294	ACGTTGGATGACAACTATATGACCCATAC	ACGTTGGATGAGACAGCGGTTTACCTTGAC	GAAGGTATTAATTTGAATGGTATTGA	116
rs2487042	ACGTTGGATGCCCTGGCATCTTCTAATTAC	ACGTTGGATGAGACAGCGGTTTACCTTGAC	GGGGAGGCCACAGCTGCCTGAAG	101
rs2487035	ACGTTGGATGCCTGGAGTTCAAATATGGTC	ACGTTGGATGAGACAGCGGTTTACCTTGAC	CACATCTGAGACAGAGAGAGA	86
rs2472516	ACGTTGGATGAGCAAAGATCCTATGTGCTA	ACGTTGGATGAGACAGCGGTTTACCTTGAC	GGAGAAAGCCAAACATCTGA	108
rs4397467	ACGTTGGATGCAAGCTAGTGAAACAGAACG	ACGTTGGATGAGACAGCGGTTTACCTTGAC	GTCTTTAGACTTGGTCTCCTC	107
rs4743776	ACGTTGGATGGAACACTAATCCTTTGTCTC	ACGTTGGATGAGACAGCGGTTTACCTTGAC	GGCACAGACTAGAAAGATA	81
rs737623	ACGTTGGATGAAAACCTCGCACCATTTGGC	ACGTTGGATGAGACAGCGGTTTACCTTGAC	GGAGGCAGGTTGTACCAA	112

Tab. 86: Applied primers for genotyping of SNPs in *ABCA1* in replication studies

SNP ID	1st Primer	2nd Primer	xt Primer	AMP_Len
rs2515606	ACGTTGGATGCAATTACCTTTGAGCCTTAC	ACGTTGGATGAGACAGCGGTTTACCTTGAC	TAGAGTGTACCCATTAAAAAAGA	105
rs2066720	ACGTTGGATGTCATTCTCTTGACATACAGG	ACGTTGGATGAGACAGCGGTTTACCTTGAC	GGGCAATTAGTCATCGAGAAGA	86

7.5.1.5 Applied Primers for Genotyping of SNPs in *LIPC*

Tab. 87: Applied primers for genotyping of SNPs in *LIPC* in initial case-control cohort from Munich

SNP ID	1st Primer	2nd Primer	xt Primer	AMP Len
rs4774297	ACGTTGGATGCAGCAGGTCATGTTGATGGG	ACGTTGGATGGTGTGACCTAGTCCTCTGTC	TTACCTAGTCCTCTGTCATCAGT	97
rs1800588	ACGTTGGATGTTGTGGTCAAAGTGTGGTGC	ACGTTGGATGAGGGCATCTTTGCTTCTTCG	TCAGCTCCTTTTGACA	95
rs17190517	ACGTTGGATGTGCAGTTTAACAAGTGTTGC	ACGTTGGATGGCTTTTCAAGCCCAAATACC	AAAAAGAAAAAATATCCCATAGAA	119
rs261341	ACGTTGGATGATGGTATATTGACTTTCATG	ACGTTGGATGCCAGGATCACTGATCTAGTC	TTATCTAGTCAAACTCCTTTATTT	113
rs483140	ACGTTGGATGCTGGTGTGGACTTACTACTC	ACGTTGGATGTTTGAGCTTCAGCAACTCTC	CCCAAGCAACTCTCCGGAAG	115
rs261336	ACGTTGGATGTGGTCTTTCGCGTTTCTGAG	ACGTTGGATGGGTTGTGATGCACACTCAAG	GAGAACCAGTGACCTAA	114
rs3825776	ACGTTGGATGAAGCCTCTTGTGTGTAGCAG	ACGTTGGATGTACTCCCACATGGTGACCAC	GGGAACCACAGTGCATGGCAATT	102
rs10518976	ACGTTGGATGCATCAGAGTATGTCCATGGC	ACGTTGGATGGGAGATGTATAGCCTTCTCA	GATCAATGTTCTATGACCTAGC	115
rs10518978	ACGTTGGATGACTGATCCATTACTTTCATC	ACGTTGGATGGACCCGAAAAGCAAAAAGCC	AGATACAAGAGTCCACAATGA	111
rs6494011	ACGTTGGATGAGCTTTGGTTCTCAAACGGG	ACGTTGGATGGAAACTCTGAGAATGAGGGC	CCCTCCACAATCTGGGTTTTAG	106
rs1968685	ACGTTGGATGCATCAGATCTTCCCTGAACC	ACGTTGGATGTGAGCACAGGGGTAAGTGGT	GTTGCTGTGGGGAACCAGAGGGGACA	111
rs1869137	ACGTTGGATGACTCTCTCATGTCACTGTCC	ACGTTGGATGATGCTCTGGGTGAGACTCAT	GGTCATCCAAGGAGTGGGACTCT	113
rs1973028	ACGTTGGATGCTGAGAACACTTGCTCAAATC	ACGTTGGATGGAAGAATGGAGAGACAGTGC	CCCAGGGAACATGCACA	118
rs2899631	ACGTTGGATGTTTTTAATTGGTGATACCAG	ACGTTGGATGGATAGGAATGCTGTCAATGG	CTATTTACCAGTCAAAATTTTAGTT	104
rs4774302	ACGTTGGATGGAGATCATTGATCATCTTGG	ACGTTGGATGAAGGGGACAGAAGAAATGCT	CCATAATAATGGTGGAAAAATATCTAC	120
rs2899632	ACGTTGGATGCCCTGTTTGCAGAGCTCTTC	ACGTTGGATGCCCCAGTCTCCAGTGAAATG	CTGCGCTATTCTGTAAATATTCAGTC	109
rs12899928	ACGTTGGATGTCAGGCAAGTCTCAAAGAAG	ACGTTGGATGTCACAGAGGTTGAGGTCATG	TCAGCTTTCCTGAGGC	116
rs4775072	ACGTTGGATGCTACTTTTAGGAAACTACAC	ACGTTGGATGGCCCTATTACAAACAATTCTC	CCCGATTACAAACAATTCTCTGATGAT	114
rs6078	ACGTTGGATGACCATCTGCCAGATCCAGTT	ACGTTGGATGAAAGGAAACTAGTGCGACCC	ACTCCCCCTCCTCAGGTGGACGGC	98
rs2242064	ACGTTGGATGGTCACTTTGAAGTTTAGCAC	ACGTTGGATGGACCATCAGGATGGTGTATG	GGATGGTGTATGGGCTTTATTT	115
rs871804	ACGTTGGATGGAGAATTGGGAAACAGTCGC	ACGTTGGATGAGGTTCCTTGTGAGAGAGTG	AAGACCTTGTGAGAGAGTGAGAATG	98
rs2414594	ACGTTGGATGAGTGGGCGAGCAAGCATAAC	ACGTTGGATGTTTGCGCTGCTATGAGGATC	CCACTGCTCTGACAGG	97
rs3829462	ACGTTGGATGGTCTCTCTCTCTAGTTTATC	ACGTTGGATGGTTCCGAGTAGTGACATGGT	GGTTGATGAACTGGATCTT	119
rs6074	ACGTTGGATGCTCATCTGATCTTTCGCTTTG	ACGTTGGATGACAGATGACCTACTACTTCG	ACCTACTACTTCGCCCAAC	111
rs4774308	ACGTTGGATGTTATCTCATCCTACCCGAGC	ACGTTGGATGGCATGGCATGGCCACAAATC	CCGCCACAAATCATTTCACC	117

7.6 Index of Figures

7.7 Index of Tables

7.8 Publications

1 S.M. Laws, K. Eckart, P. Friedrich, A. Kurz, H. Förstl and M. Riemenschneider; Lack of evidence to support the association of polymorphisms within the alpha- and beta-secretase genes (ADAM10/BACE1) with Alzheimer's disease; *Neurobiol Aging 2009*

2 S.M. Laws, K. Eckart, P. Friedrich, T. Eisele, A. Kurz, H. Förstl and M. Riemenschneider; No association of lipase C polymorphisms with Alzheimer's disease; *Neurobiol Aging 2008*

3 P. Friedrich; T.M. Feulner; S.M. Laws; K. Eckart; R. Perneczky; A. Kurz; H. Förstl; M. Riemenschneider; No association of Tachykinin receptor 2 (TACR2) polymorphisms with Alzheimer's disease; *Neurobiol Aging 2008*

4 K. Eckart, S.M. Laws, P. Friedrich, S. Wagenpfeil, C. Riehle, T. Eisele, J. Müller, R.M. Page, C. Haass, C. Graff, B.F. Björk, H. Förstl, A. Kurz and M. Riemenschneider; Common APP variants are associated with Alzheimer's disease and cause alterations of gene expression and APP processing; *to be submitted soon*

7.9 Curriculum Vitae

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since 05/2005	Ph.D. student, Klinik und Poliklinik für Psychiatrie und Psychotherapie, Klinikum rechts der Isar, Technische Universität München
11/2003 – 07/2004	Post-graduate programme English-speaking countries in conjunction with general & business English (ESL), Ludwig-Maximilians-Universität, München
06/2003	Graduation to Dipl.-Biol. univ. (biology diploma: plantphysiology, zoology, anorganic chemistry, organic chemistry, physical chemistry)
1996 – 2003	Study of chemistry and biology at Ludwig-Maximilians-Universität, München
06/1996	Graduation with "Allgemeine Hochschulreife" (Abitur) from Georg-Wilhelm-Steller-Gymnasium, Bad Windsheim
1983 –1996	Primary and secondary school education

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7.10 References

Ando, T., S. S. Monroe, et al. (1997). "A one-tube method of reverse transcription-PCR to efficiently amplify a 3-kilobase region from the RNA polymerase gene to the poly(A) tail of small round-structured viruses (Norwalk-like viruses)." J Clin Microbiol **35**(3): 570-7.

Athan, E. S., J. H. Lee, et al. (2002). "Polymorphisms in the promoter of the human APP gene: functional evaluation and allele frequencies in Alzheimer disease." Arch Neurol **59**(11): 1793-9.

Balistreri, C. R., M. P. Grimaldi, et al. (2006). "Association between the polymorphism of CCR5 and Alzheimer's disease: results of a study performed on male and female patients from Northern Italy." Ann N Y Acad Sci **1089**: 454-61.

Baroni, M. G., A. Berni, et al. (2003). "Genetic study of common variants at the Apo E, Apo AI, Apo CIII, Apo B, lipoprotein lipase (LPL) and hepatic lipase (LIPC) genes and coronary artery disease (CAD): variation in LIPC gene associates with clinical outcomes in patients with established CAD." BMC Med Genet **4**: 8.

Barrachina, M., T. Maes, et al. (2006). "Lysosome-associated membrane protein 1 (LAMP-1) in Alzheimer's disease." Neuropathol Appl Neurobiol **32**(5): 505-16.

Barrett, J. C., B. Fry, et al. (2005). "Haploview: analysis and visualization of LD and haplotype maps." Bioinformatics **21**(2): 263-5.

Bassiony, M. M., A. Rosenblatt, et al. (2004). "Falls and age in patients with Alzheimer's disease." J Nerv Ment Dis **192**(8): 570-2.

Bauer, J., M. Hull, et al. (1995). "[Pathogenetic factors of Alzheimer disease]." Z Gerontol Geriatr **28**(3): 155.

Becaria, A., A. Campbell, et al. (2002). "Aluminum as a toxicant." Toxicol Ind Health **18**(7): 309-20.

Bertram, L., D. Blacker, et al. (2000). "Evidence for genetic linkage of Alzheimer's disease to chromosome 10q." Science **290**(5500): 2302-3.

Bertram, L., M. B. McQueen, et al. (2007). "Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database." Nat Genet **39**(1): 17-23.

Bickeboller, H. and C. Fischer (2007). Einführung in die Genetische Epidemiologie, Springer.

Bickel, H. (2000). "[Dementia syndrome and Alzheimer disease: an assessment of morbidity and annual incidence in Germany]." Gesundheitswesen **62**(4): 211-8.

Bickel, H. (2001). "[Dementia in advanced age: estimating incidence and health care costs]." Z Gerontol Geriatr **34**(2): 108-15.

Blacker, D., L. Bertram, et al. (2003). "Results of a high-resolution genome screen of 437 Alzheimer's disease families." Hum Mol Genet **12**(1): 23-32.

Bleck, O., J. A. McGrath, et al. (2001). "Searching for candidate genes in the new millennium." Clin Exp Dermatol **26**(3): 279-83.

Bodzioch, M., E. Orso, et al. (1999). "The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease." Nat Genet **22**(4): 347-51.

Bouwman, F. H., N. S. Schoonenboom, et al. (2008). "CSF biomarker levels in early and late onset Alzheimer's disease." Neurobiol Aging.

Brooks-Wilson, A., M. Marcil, et al. (1999). "Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency." Nat Genet **22**(4): 336-45.

Brown, R. C., A. H. Lockwood, et al. (2005). "Neurodegenerative diseases: an overview of environmental risk factors." Environ Health Perspect **113**(9): 1250-6.

Buee, L., T. Bussiere, et al. (2000). "Tau protein isoforms, phosphorylation and role in neurodegenerative disorders." Brain Res Brain Res Rev **33**(1): 95-130.

Cai, S. J., D. M. Wong, et al. (1989). "Structure of the human hepatic triglyceride lipase gene." Biochemistry **28**(23): 8966-71.

Candore, G., C. R. Balistreri, et al. (2006). "Age-related inflammatory diseases: role of genetics and gender in the pathophysiology of Alzheimer's disease." Ann N Y Acad Sci **1089**: 472-86.

Canevari, L. and J. B. Clark (2007). "Alzheimer's disease and cholesterol: the fat connection." Neurochem Res **32**(4-5): 739-50.

Chu, L. W., Y. Li, et al. (2007). "A novel intronic polymorphism of ABCA1 gene reveals risk for sporadic Alzheimer's disease in Chinese." Am J Med Genet B Neuropsychiatr Genet **144B**(8): 1007-13.

Colucci, M., S. Cammarata, et al. (2006). "The number of pregnancies is a risk factor for Alzheimer's disease." Eur J Neurol **13**(12): 1374-7.

Combs, C. K., P. Bates, et al. (2001). "Regulation of beta-amyloid stimulated proinflammatory responses by peroxisome proliferator-activated receptor alpha." Neurochem Int **39**(5-6): 449-57.

Coon, K. D., A. J. Myers, et al. (2007). "A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease." J Clin Psychiatry **68**(4): 613-8.

Corder, E. H., E. Ghebremedhin, et al. (2004). "The biphasic relationship between regional brain senile plaque and neurofibrillary tangle distributions: modification by age, sex, and APOE polymorphism." Ann N Y Acad Sci **1019**: 24-8.

Cribbs, D. H., W. W. Poon, et al. (2004). "Caspase-mediated degeneration in Alzheimer's disease." Am J Pathol **165**(2): 353-5.

Crowther, M. A. and D. J. Cook (2007). "Trials and tribulations of systematic reviews and meta-analyses." Hematology Am Soc Hematol Educ Program **2007**: 493-7.

Cruts, M., H. Backhovens, et al. (1995). "Molecular genetic analysis of familial early-onset Alzheimer's disease linked to chromosome 14q24.3." Hum Mol Genet **4**(12): 2363-71.

Davis, J., M. R. Wagner, et al. (2003). "Amyloid beta-protein stimulates the expression of urokinase-type plasminogen activator (uPA) and its receptor (uPAR) in human cerebrovascular smooth muscle cells." J Biol Chem **278**(21): 19054-61.

Daw, E. W., H. Payami, et al. (2000). "The number of trait loci in late-onset Alzheimer disease." Am J Hum Genet **66**(1): 196-204.

De Jager, C. A., E. Hogervorst, et al. (2003). "Sensitivity and specificity of neuropsychological tests for mild cognitive impairment, vascular cognitive impairment and Alzheimer's disease." Psychol Med **33**(6): 1039-50.

de la Chapelle, A. and F. A. Wright (1998). "Linkage disequilibrium mapping in isolated populations: the example of Finland revisited." Proc Natl Acad Sci U S A **95**(21): 12416-23.

De Leon, M. J., A. E. George, et al. (1997). "Frequency of hippocampal formation atrophy in normal aging and Alzheimer's disease." Neurobiol Aging **18**(1): 1-11.

Dickson, D. W. (2004). "Apoptotic mechanisms in Alzheimer neurofibrillary degeneration: cause or effect?" J Clin Invest **114**(1): 23-7.

Edbauer, D., E. Winkler, et al. (2003). "Reconstitution of gamma-secretase activity." Nat Cell Biol **5**: 486-488.

Ehehalt, R., B. Michel, et al. (2002). "Splice variants of the beta-site APP-cleaving enzyme BACE1 in human brain and pancreas." Biochem Biophys Res Commun **293**(1): 30-7.

Feulner, T. M., S. M. Laws, et al. (2009). "Examination of the current top candidate genes for AD in a genome-wide association study." Mol Psychiatry.

Folstein, M. F., S. E. Folstein, et al. (1975). "'Mini Mental State'. A practical method for grading the cognitive state of patients for the clinician." J Psychiat Res **12**: 189-198.

Forstl, H. (2000). "Clinical issues in current drug therapy for dementia." Alzheimer Dis Assoc Disord **14 Suppl 1**: S103-8.

Gabriel, S. B., S. F. Schaffner, et al. (2002). "The structure of haplotype blocks in the human genome." Science **296**(5576): 2225-9.

Garcia, T. and D. Jay (2004). "[Phosphorylation of tau and Alzheimer's disease]." Gac Med Mex **140**(3): 329-33.

Gasparini, L., W. J. Netzer, et al. (2002). "Does insulin dysfunction play a role in Alzheimer's disease?" Trends Pharmacol Sci **23**(6): 288-93.

Goate, A., M. C. Chartier-Harlin, et al. (1991). "Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease." Nature **349**(6311): 704-6.

Goate, A. M., A. R. Haynes, et al. (1989). "Predisposing locus for Alzheimer's disease on chromosome 21." Lancet **1**(8634): 352-5.

Gomez-Ramos, A., M. A. Smith, et al. (2004). "Tau phosphorylation and assembly." Acta Neurobiol Exp (Wars) **64**(1): 33-9.

Gong, C. X., F. Liu, et al. (2005). "Post-translational modifications of tau protein in Alzheimer's disease." J Neural Transm **112**(6): 813-38.

Griffin, T. J. and L. M. Smith (2000). "Genetic identification by mass spectrometric analysis of single-nucleotide polymorphisms: ternary encoding of genotypes." Anal Chem **72**(14): 3298-302.

Grossberg, G. T. (2005). "Effect of rivastigmine in the treatment of behavioral disturbances associated with dementia: review of neuropsychiatric impairment in Alzheimer's disease." Curr Med Res Opin **21**(10): 1631-9.

Grossman, H., C. Bergmann, et al. (2006). "Dementia: a brief review." Mt Sinai J Med **73**(7): 985-92.

Gut, I. G. (2001). "Automation in genotyping of single nucleotide polymorphisms." Hum Mutat **17**(6): 475-92.

Guyant-Marechal, L., A. Rovelet-Lecrux, et al. (2007). "Variations in the APP gene promoter region and risk of Alzheimer disease." Neurology **68**(9): 684-7.

Haniu, M., P. Denis, et al. (2000). "Characterization of Alzheimer's beta -secretase protein BACE. A pepsin family member with unusual properties." J Biol Chem **275**(28): 21099-106.

Hardy, J. and D. J. Selkoe (2002). "The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics." Science **297**(5580): 353-6.

Hardy, J. A. and G. A. Higgins (1992). "Alzheimer's disease: the amyloid cascade hypothesis." Science **256**(5054): 184-5.

Hartmann, A. P., S. M. Almeida, et al. (2004). "Hyperphosphorylated tau protein in the cerebrospinal fluid of patients with Alzheimer's disease and other dementias: preliminary findings." Arq Neuropsiquiatr **62**(3B): 751-5.

Hellstrom-Lindahl, E., M. Mousavi, et al. (2004). "Reduced levels of Abeta 40 and Abeta 42 in brains of smoking controls and Alzheimer's patients." Neurobiol Dis **15**(2): 351-60.

Hillenkamp, F., M. Karas, et al. (1991). "Matrix-assisted laser desorption/ionization mass spectrometry of biopolymers." Anal Chem **63**(24): 1193A-1203A.

Hirsch-Reinshagen, V., B. L. Burgess, et al. (2008). "Why lipids are important for Alzheimer disease?" Mol Cell Biochem.

Huang, W., C. Qiu, et al. (2004). "APOE genotype, family history of dementia, and Alzheimer disease risk: a 6-year follow-up study." Arch Neurol **61**(12): 1930-4.

Itzhaki, R. (2004). "Herpes simplex virus type 1, apolipoprotein E and Alzheimer' disease." Herpes **11 Suppl 2**: 77A-82A.

Itzhaki, R. F., C. B. Dobson, et al. (2004). "The role of viruses and of APOE in dementia." Ann N Y Acad Sci **1019**: 15-8.

Jarvenpaa, T., J. O. Rinne, et al. (2002). "Characteristics of two telephone screens for cognitive impairment." Dement Geriatr Cogn Disord **13**(3): 149-55.

Jellinger, K., W. Danielczyk, et al. (1990). "Clinicopathological analysis of dementia disorders in the elderly." J Neurol Sci **95**(3): 239-58.

Jorm, A. F., R. Scott, et al. (1988). "Educational level differences on the Mini-Mental State: the role of test bias." Psychol Med **18**(3): 727-31.

Juan, D., D. H. Zhou, et al. (2004). "A 2-year follow-up study of cigarette smoking and risk of dementia." Eur J Neurol **11**(4): 277-82.

Karas, M. and F. Hillenkamp (1988). "Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons." Anal Chem **60**(20): 2299-301.

Karp, A., I. Kareholt, et al. (2004). "Relation of education and occupation-based socioeconomic status to incident Alzheimer's disease." Am J Epidemiol **159**(2): 175-83.

Kathiresan, S., O. Melander, et al. (2008). "Polymorphisms associated with cholesterol and risk of cardiovascular events." N Engl J Med **358**(12): 1240-9.

Katzov, H., K. Chalmers, et al. (2004). "Genetic variants of ABCA1 modify Alzheimer disease risk and quantitative traits related to beta-amyloid metabolism." Hum Mutat **23**(4): 358-67.

Kehoe, P., F. Wavrant-De Vrieze, et al. (1999). "A full genome scan for late onset Alzheimer's disease." Hum Mol Genet **8**(2): 237-45.

Kirpekar, F., E. Nordhoff, et al. (1998). "DNA sequence analysis by MALDI mass spectrometry." Nucleic Acids Res **26**(11): 2554-9.

Knoblauch, H., A. Bauerfeind, et al. (2004). "Haplotypes and SNPs in 13 lipid-relevant genes explain most of the genetic variance in high-density lipoprotein and low-density lipoprotein cholesterol." Hum Mol Genet **13**(10): 993-1004.

Kojro, E. and F. Fahrenholz (2005). "The non-amyloidogenic pathway: structure and function of alpha-secretases." Subcell Biochem **38**: 105-27.

Koldamova, R., M. Staufenbiel, et al. (2005). "Lack of ABCA1 considerably decreases brain ApoE level and increases amyloid deposition in APP23 mice." J Biol Chem **280**(52): 43224-35.

Koldamova, R. P., I. M. Lefterov, et al. (2003). "22R-hydroxycholesterol and 9-cis-retinoic acid induce ATP-binding cassette transporter A1 expression and cholesterol efflux in brain cells and decrease amyloid beta secretion." J Biol Chem **278**(15): 13244-56.

Kolsch, H., D. Lutjohann, et al. (2006). "Polymorphism in ABCA1 influences CSF 24S-hydroxycholesterol levels but is not a major risk factor of Alzheimer's disease." Int J Mol Med **17**(5): 791-4.

Kowalska, A., D. Pruchnik-Wolinska, et al. (2004). "Genetic study of familial cases of Alzheimer's disease." Acta Biochim Pol **51**(1): 245-52.

Kowalska, A., D. Pruchnik-Wolinska, et al. (2004). "Amyloid precursor protein gene analysis in familial Alzheimer's disease cases: a lack of mutations in exons 16 and 17." Folia Neuropathol **42**(1): 1-7.

Lahiri, D. K., Y. W. Ge, et al. (2005). "Characterization of the APP proximal promoter and 5'-untranslated regions: identification of cell type-specific domains and implications in APP gene expression and Alzheimer's disease." FASEB J **19**(6): 653-5.

Laird, N. M., S. Horvath, et al. (2000). "Implementing a unified approach to family-based tests of association." Genet Epidemiol **19 Suppl 1**: S36-42.

Lamb, B. T., S. S. Sisodia, et al. (1993). "Introduction and expression of the 400 kilobase amyloid precursor protein gene in transgenic mice [corrected]." Nat Genet **5**(1): 22-30.

Lammich, S., E. Kojro, et al. (1999). "Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease." Proc Natl Acad Sci U S A **96**(7): 3922-7.

Laws, S. M., K. Eckart, et al. (2008). "No association of lipase C polymorphisms with Alzheimer's disease." Neurobiol Aging **NBA-S-08-00664-1**.

Laws, S. M., K. Eckart, et al. (2009). "Lack of evidence to support the association of polymorphisms within the alpha- and beta-secretase genes (ADAM10/BACE1) with Alzheimer's disease." Neurobiol Aging **NBA-08-634R1**.

Lee, J. H., R. Cheng, et al. (2006). "Expanded genomewide scan implicates a novel locus at 3q28 among Caribbean hispanics with familial Alzheimer disease." Arch Neurol **63**(11): 1591-8.

Letenneur, L. (2004). "Risk of dementia and alcohol and wine consumption: a review of recent results." Biol Res **37**(2): 189-93.

Letenneur, L., S. Larrieu, et al. (2004). "Alcohol and tobacco consumption as risk factors of dementia: a review of epidemiological studies." Biomed Pharmacother **58**(2): 95-9.

Levy-Lahad, E., E. M. Wijsman, et al. (1995). "A familial Alzheimer's disease locus on chromosome 1." Science **269**(5226): 970-3.

Lewis, C. M. (2002). "Genetic association studies: design, analysis and interpretation." Brief Bioinform **3**(2): 146-53.

Li, H., S. Wetten, et al. (2008). "Candidate single-nucleotide polymorphisms from a genomewide association study of Alzheimer disease." Arch Neurol **65**(1): 45-53.

Li, L., R. Perry, et al. (1998). "Polymorphic tetranucleotide repeat site within intron 7 of the beta-amyloid precursor protein gene and its lack of association with Alzheimer's disease." Hum Genet **103**(1): 86-9.

Li, Y., K. Tacey, et al. (2004). "Association of ABCA1 with late-onset Alzheimer's disease is not observed in a case-control study." Neurosci Lett **366**(3): 268-71.

Ling, Y., K. Morgan, et al. (2003). "Amyloid precursor protein (APP) and the biology of proteolytic processing: relevance to Alzheimer's disease." Int J Biochem Cell Biol **35**(11): 1505-35.

Lottspeich, F. (1998). Bioanalytik, Spektrum Verlag.

Lovestone, S. and C. H. Reynolds (1997). "The phosphorylation of tau: a critical stage in neurodevelopment and neurodegenerative processes." Neuroscience **78**(2): 309-24.

Luchsinger, J. A. and R. Mayeux (2004). "Dietary factors and Alzheimer's disease." Lancet Neurol **3**(10): 579-87.

Luchsinger, J. A., M. X. Tang, et al. (2004). "Hyperinsulinemia and risk of Alzheimer disease." Neurology **63**(7): 1187-92.

Luciani, M. F., F. Denizot, et al. (1994). "Cloning of two novel ABC transporters mapping on human chromosome 9." Genomics **21**(1): 150-9.

Luo, Y., B. Bolon, et al. (2001). "Mice deficient in BACE1, the Alzheimer's beta-secretase, have normal phenotype and abolished beta-amyloid generation." Nat Neurosci **4**(3): 231-2.

Mahley, R. W. and S. C. Rall, Jr. (2000). "Apolipoprotein E: far more than a lipid transport protein." Annu Rev Genomics Hum Genet **1**: 507-37.

Malhotra, K., L. Foltz, et al. (1998). "Interaction and effect of annealing temperature on primers used in differential display RT-PCR." Nucleic Acids Res **26**(3): 854-6.

Martins, I. J., E. Hone, et al. (2006). "Apolipoprotein E, cholesterol metabolism, diabetes, and the convergence of risk factors for Alzheimer's disease and cardiovascular disease." Mol Psychiatry **11**(8): 721-36.

Martins, R. N., R. Clarnette, et al. (1995). "ApoE genotypes in Australia: roles in early and late onset Alzheimer's disease and Down's syndrome." Neuroreport **6**(11): 1513-6.

Matsui, T., M. Ingelsson, et al. (2007). "Expression of APP pathway mRNAs and proteins in Alzheimer's disease." Brain Res **1161**: 116-23.

Melchor, J. P., R. Pawlak, et al. (2003). "The tissue plasminogen activator-plasminogen proteolytic cascade accelerates amyloid-beta (Abeta) degradation and inhibits Abeta-induced neurodegeneration." J Neurosci **23**(26): 8867-71.

Merched, A., Y. Xia, et al. (2000). "Decreased high-density lipoprotein cholesterol and serum apolipoprotein AI concentrations are highly correlated with the severity of Alzheimer's disease." Neurobiol Aging **21**(1): 27-30.

Mueller, J. C., E. Lohmussaar, et al. (2005). "Linkage disequilibrium patterns and tagSNP transferability among European populations." Am J Hum Genet **76**(3): 387-98.

Myers, A., P. Holmans, et al. (2000). "Susceptibility locus for Alzheimer's disease on chromosome 10." Science **290**(5500): 2304-5.

Myers, A., F. Wavrant De-Vrieze, et al. (2002). "Full genome screen for Alzheimer disease: stage II analysis." Am J Med Genet **114**(2): 235-44.

Nagy, Z., N. J. Hindley, et al. (1999). "The progression of Alzheimer's disease from limbic regions to the neocortex: clinical, radiological and pathological relationships." Dement Geriatr Cogn Disord **10**(2): 115-20.

Nechiporuk, A., P. Fain, et al. (1993). "Linkage of familial Alzheimer disease to chromosome 14 in two large early-onset pedigrees: effects of marker allele frequencies on lod scores." Am J Med Genet **48**(1): 63-6.

Nowotny, P., X. Simcock, et al. (2007). "Association studies testing for risk for late-onset Alzheimer's disease with common variants in the beta-amyloid precursor protein (APP)." Am J Med Genet B Neuropsychiatr Genet **144B**(4): 469-74.

Olarte, L., N. Schupf, et al. (2006). "Apolipoprotein E epsilon4 and age at onset of sporadic and familial Alzheimer disease in Caribbean Hispanics." Arch Neurol **63**(11): 1586-90.

Olson, J. M., K. A. Goddard, et al. (2002). "A second locus for very-late-onset Alzheimer disease: a genome scan reveals linkage to 20p and epistasis between 20p and the amyloid precursor protein region." Am J Hum Genet **71**(1): 154-61.

Oram, J. F. and R. M. Lawn (2001). "ABCA1. The gatekeeper for eliminating excess tissue cholesterol." J Lipid Res **42**(8): 1173-9.

Ott, A., K. Andersen, et al. (2004). "Effect of smoking on global cognitive function in nondemented elderly." Neurology **62**(6): 920-4.

Ott, A., A. J. Slooter, et al. (1998). "Smoking and risk of dementia and Alzheimer's disease in a population-based cohort study: the Rotterdam Study." Lancet **351**(9119): 1840-3.

Ozturk, A., R. L. Minster, et al. (2007). "Association of tagSNPs in the urokinase-plasminogen activator (PLAU) gene with Alzheimer's disease and associated quantitative traits." Am J Med Genet B Neuropsychiatr Genet **144B**(1): 79-82.

Pantel, J., P. Schonknecht, et al. (2004). "[Neuropsychological correlates of cerebral atrophy in Alzheimer's dementia]." Fortschr Neurol Psychiatr **72**(4): 192-203.

Pastor, P. and A. M. Goate (2004). "Molecular genetics of Alzheimer's disease." Curr Psychiatry Rep **6**(2): 125-33.

Pearson, J. V., M. J. Huentelman, et al. (2007). "Identification of the genetic basis for complex disorders by use of pooling-based genomewide single-nucleotide-polymorphism association studies." Am J Hum Genet **80**(1): 126-39.

Pericak-Vance, M. A., M. L. Bass, et al. (1998). "Complete genomic screen in late-onset familial Alzheimer's disease." Neurobiol Aging **19**(1 Suppl): S39-42.

Perry, G., L. M. Sayre, et al. (2002). "The role of iron and copper in the aetiology of neurodegenerative disorders: therapeutic implications." CNS Drugs **16**(5): 339-52.

Pfaffl, M. W. (2004). BIOspektrum, Sonderausgabe PCR

Postina, R. (2008). "A closer look at alpha-secretase." Curr Alzheimer Res **5**(2): 179-86.

Postina, R., A. Schroeder, et al. (2004). "A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model." J Clin Invest **113**(10): 1456-64.

Potyk, D. (2005). "Treatments for Alzheimer disease." South Med J **98**(6): 628-35.

Prasher, V. P., M. J. Farrer, et al. (1998). "Molecular mapping of Alzheimer-type dementia in Down's syndrome." Ann Neurol **43**(3): 380-3.

Priller, C., T. Bauer, et al. (2006). "Synapse formation and function is modulated by the amyloid precursor protein." J Neurosci **26**(27): 7212-21.

Prinzen, C., U. Muller, et al. (2005). "Genomic structure and functional characterization of the human ADAM10 promoter." FASEB J **19**(11): 1522-4.

Qi-Takahara, Y., M. Morishima-Kawashima, et al. (2005). "Longer forms of amyloid beta protein: implications for the mechanism of intramembrane cleavage by gamma-secretase." J Neurosci **25**(2): 436-45.

Qiu, C., A. Karp, et al. (2003). "Lifetime principal occupation and risk of Alzheimer's disease in the Kungsholmen project." Am J Ind Med **43**(2): 204-11.

Rademakers, R., M. Cruts, et al. (2003). "Genetics of early-onset Alzheimer dementia." ScientificWorldJournal **3**: 497-519.

Rebeck, G. W., M. J. LaDu, et al. (2006). "The generation and function of soluble apoE receptors in the CNS." Mol Neurodegener **1**: 15.

Reid, P. C., Y. Urano, et al. (2007). "Alzheimer's disease: cholesterol, membrane rafts, isoprenoids and statins." J Cell Mol Med **11**(3): 383-92.

Reiman, E. M., J. A. Webster, et al. (2007). "GAB2 alleles modify Alzheimer's risk in APOE epsilon4 carriers." Neuron **54**(5): 713-20.

Riemenschneider, M., K. Blennow, et al. (2006). "The cathepsin D rs17571 polymorphism: effects on CSF tau concentrations in Alzheimer disease." Hum Mutat **27**(6): 532-7.

Rissman, R. A., W. W. Poon, et al. (2004). "Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology." J Clin Invest **114**(1): 121-30.

Rodriguez-Rodriguez, E., I. Mateo, et al. (2007). "Association of genetic variants of ABCA1 with Alzheimer's disease risk." Am J Med Genet B Neuropsychiatr Genet **144B**(7): 964-8.

Rosler, N., I. Wichart, et al. (2001). "CSF Abeta40 and Abeta42: Natural course and clinical usefulness." J Alzheimers Dis **3**(6): 599-600.

Rostoks, N., S. Mudie, et al. (2005). "Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress." Mol Genet Genomics **274**(5): 515-27.

Rottkamp, C. A., A. K. Raina, et al. (2001). "Redox-active iron mediates amyloid-beta toxicity." Free Radic Biol Med **30**(4): 447-50.

Rovelet-Lecrux, A., D. Hannequin, et al. (2006). "APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy." Nat Genet **38**(1): 24-6.

Ruitenberg, A., J. C. van Swieten, et al. (2002). "Alcohol consumption and risk of dementia: the Rotterdam Study." Lancet **359**(9303): 281-6.

Rumble, B., R. Retallack, et al. (1989). "Amyloid A4 protein and its precursor in Down's syndrome and Alzheimer's disease." N Engl J Med **320**(22): 1446-52.

Rust, S., M. Rosier, et al. (1999). "Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1." Nat Genet **22**(4): 352-5.

Saiki, R. K., D. H. Gelfand, et al. (1988). "Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase." Science **239**(4839): 487-91.

Santamarina-Fojo, S., K. Peterson, et al. (2000). "Complete genomic sequence of the human ABCA1 gene: analysis of the human and mouse ATP-binding cassette A promoter." Proc Natl Acad Sci U S A **97**(14): 7987-92.

Saunders, A. M., W. J. Strittmatter, et al. (1993). "Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease." Neurology **43**(8): 1467-72.

Schmitz, G., W. E. Kaminski, et al. (2000). "ABC transporters in cellular lipid trafficking." Curr Opin Lipidol **11**(5): 493-501.

Schork, N. J., D. Fallin, et al. (2001). "The future of genetic case-control studies." Adv Genet **42**: 191-212.

Schupf, N., B. Patel, et al. (2001). "Elevated plasma amyloid beta-peptide 1-42 and onset of dementia in adults with Down syndrome." Neurosci Lett **301**(3): 199-203.

Scott, W. K., E. R. Hauser, et al. (2003). "Ordered-subsets linkage analysis detects novel Alzheimer disease loci on chromosomes 2q34 and 15q22." Am J Hum Genet **73**(5): 1041-51.

Sennvik, K., N. Bogdanovic, et al. (2004). "Beta-secretase-cleaved amyloid precursor protein in Alzheimer brain: a morphologic study." J Cell Mol Med **8**(1): 127-34.

SEQUENOM, i. G. A., November 2006.

Sergeant, N., A. Delacourte, et al. (2005). "Tau protein as a differential biomarker of tauopathies." Biochim Biophys Acta **1739**(2-3): 179-97.

Shibata, N., T. Kawarai, et al. (2006). "Association studies of cholesterol metabolism genes (CH25H, ABCA1 and CH24H) in Alzheimer's disease." Neurosci Lett **391**(3): 142-6.

Smith, A. D. and K. A. Jobst (1996). "Use of structural imaging to study the progression of Alzheimer's disease." Br Med Bull **52**(3): 575-86.

Smyth, K. A., T. Fritsch, et al. (2004). "Worker functions and traits associated with occupations and the development of AD." Neurology **63**(3): 498-503.

Sparks, D. L., S. W. Scheff, et al. (1994). "Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol." Exp Neurol **126**(1): 88-94.

Spielman, R. S. and W. J. Ewens (1998). "A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test." Am J Hum Genet **62**(2): 450-8.

Spielman, R. S., R. E. McGinnis, et al. (1993). "Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM)." Am J Hum Genet **52**(3): 506-16.

Stern, Y., B. Gurland, et al. (1994). "Influence of education and occupation on the incidence of Alzheimer's disease." Jama **271**(13): 1004-10.

Strittmatter, W. J., K. H. Weisgraber, et al. (1993). "Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease." Proc Natl Acad Sci U S A **90**(17): 8098-102.

Tanahashi, H. and T. Tabira (2001). "Three novel alternatively spliced isoforms of the human beta-site amyloid precursor protein cleaving enzyme (BACE) and their effect on amyloid beta-peptide production." Neurosci Lett **307**(1): 9-12.

Teri, L., S. M. McCurry, et al. (1997). "Memory, thinking, and aging. What we know about what we know." West J Med **167**(4): 269-75.

Theuns, J., N. Brouwers, et al. (2006). "Promoter mutations that increase amyloid precursor-protein expression are associated with Alzheimer disease." Am J Hum Genet **78**(6): 936-46.

Todd, S., A. J. McKnight, et al. (2008). "BACE1 Polymorphisms Do Not Influence Platelet Membrane beta-secretase Activity or Genetic Susceptibility for Alzheimer's Disease in the Northern Irish Population." Neuromolecular Med.

Truelsen, T., D. Thudium, et al. (2002). "Amount and type of alcohol and risk of dementia: the Copenhagen City Heart Study." Neurology **59**(9): 1313-9.

Turner, P. R., K. O'Connor, et al. (2003). "Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory." Prog Neurobiol **70**(1): 1-32.

van Duijn, C. M. and A. Hofman (1991). "Relation between nicotine intake and Alzheimer's disease." BMJ **302**(6791): 1491-4.

Vanderstichele, H., E. Van Kerschaver, et al. (2000). "Standardization of measurement of beta-amyloid(1-42) in cerebrospinal fluid and plasma." Amyloid **7**(4): 245-58.

Vassar, R., B. Bennett, et al. (1999). "b-secretase cleavage of Alzheimer´s Amyloid precursor protein by the transmembrane aspartic protease BACE." Science **286**: 735-741.

Vassar, R., B. D. Bennett, et al. (1999). "Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE." Science **286**(5440): 735-41.

Verdile, G., S. Fuller, et al. (2004). "The role of beta amyloid in Alzheimer's disease: still a cause of everything or the only one who got caught?" Pharmacol Res **50**(4): 397-409.

von Arnim, C. A., A. Kinoshita, et al. (2005). "The low density lipoprotein receptor-related protein (LRP) is a novel beta-secretase (BACE1) substrate." J Biol Chem **280**(18): 17777-85.

Wahrle, S. E., H. Jiang, et al. (2008). "Overexpression of ABCA1 reduces amyloid deposition in the PDAPP mouse model of Alzheimer disease." J Clin Invest **118**(2): 671-82.

Wahrle, S. E., A. R. Shah, et al. (2007). "Apolipoprotein E levels in cerebrospinal fluid and the effects of ABCA1 polymorphisms." Mol Neurodegener **2**: 7.

Wang, F. and J. P. Jia (2007). "[Correlation of cholesterol 24-hydroxylase and ATP-binding cassette transporter A1 polymorphisms with Alzheimer's disease]." Zhonghua Yi Xue Za Zhi **87**(9): 614-8.

Wang, N. and A. R. Tall (2003). "Regulation and mechanisms of ATP-binding cassette transporter A1-mediated cellular cholesterol efflux." Arterioscler Thromb Vasc Biol **23**(7): 1178-84.

Wang, S. C., B. Oelze, et al. (2008). "Age-specific epigenetic drift in late-onset Alzheimer's disease." PLoS ONE **3**(7): e2698.

Whitehouse, P. J. (1987). "Neurotransmitter receptor alterations in Alzheimer disease: a review." Alzheimer Dis Assoc Disord **1**(1): 9-18.

Whittemore, A. S. and I. P. Tu (2000). "Detection of disease genes by use of family data. I. Likelihood-based theory." Am J Hum Genet **66**(4): 1328-40.

Willem, M., A. N. Garratt, et al. (2006). "Control of peripheral nerve myelination by the beta-secretase BACE1." Science **314**(5799): 664-6.

Wolfsberg, T. G., P. Primakoff, et al. (1995). "ADAM, a novel family of membrane proteins containing A Disintegrin And Metalloprotease domain: multipotential functions in cell-cell and cell-matrix interactions." J Cell Biol **131**(2): 275-8.

Wollmer, M. A., K. Sleegers, et al. (2007). "Association study of cholesterol-related genes in Alzheimer's disease." Neurogenetics **8**(3): 179-88.

Wollmer, M. A., J. R. Streffer, et al. (2003). "ABCA1 modulates CSF cholesterol levels and influences the age at onset of Alzheimer's disease." Neurobiol Aging **24**(3): 421-6.

Xu, W. L., C. X. Qiu, et al. (2004). "Diabetes mellitus and risk of dementia in the Kungsholmen project: a 6-year follow-up study." Neurology **63**(7): 1181-6.

Yoshikai, S., H. Sasaki, et al. (1990). "Genomic organization of the human amyloid beta-protein precursor gene." Gene **87**(2): 257-63.

Zekanowski, C., D. Religa, et al. (2004). "Genetic aspects of Alzheimer's disease." Acta Neurobiol Exp (Wars) **64**(1): 19-31.

Zheng, H. and E. H. Koo (2006). "The amyloid precursor protein: beyond amyloid." Mol Neurodegener **1**: 5.

Zhu, H., J. W. Taylor, et al. (2008). "Lack of association of hepatic lipase polymorphisms with late-onset Alzheimer's disease." Neurobiol Aging **29**(5): 793-4.