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„Given and acquired risk factors in cognitive decline, the development of Mild Cognitive Impairment, and Alzheimer's Disease: results of a prospective, population-based, longitudinal study"

presented by
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List of publications for the publication based dissertation

I. Publication

Toro, P., **Degen, C**., Pierer, M., Gustafson, D., Schröder, J., & Schönknecht, P. (2014). Cholesterol in Mild Cognitive Impairment and Alzheimer's disease in a birth cohort over 14 years. *European Archives of Psychiatry and Clinical Neuroscience, 264*, 485-492.

II. Publication

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III. Publication

Degen, C., Toro, P., Schönknecht, P., Sattler, C., Schröder, J. (2016). Diabetes mellitus type II and cognitive capacity in healthy aging and Mild Cognitive Impairment. *Psychiatry Research*, *240*, 42-46.

1. General Introduction

Alzheimer's Disease (AD) and Mild Cognitive Impairment (MCI) as its prodromal stage are accompanied by structural and functional cerebral changes that often occur before the onset of symptoms. It is therefore assumed that disease-related processes are taking place long before symptoms become apparent rendering primary and secondary preventive measures inapt. While advances in clinical practice, including the use of novel imaging techniques (e.g. amyloid positron emission tomography) and/or identification of disease-related biomarkers (e.g. β-amyloid and τ-protein) have enabled physicians to detect disease progression at relatively early stages, the identification of genetic and acquired risk factors in developing MCI/AD could be of great prognostic value even before disease onset and may bear important implications for disease prevention and intervention, e.g. through the adaptation of lifestyle factors and early implementation of adequate training approaches.

While studies have shown that early-onset AD (before the age of 65) has a strong genetic component, the exact role of genetic and acquired risk factors in the development of late-onset (or sporadic) AD or MCI remains a subject of zealous research and results remain inconclusive. Thus, when evaluating one's own risk of developing MCI/AD, genetic counseling - including susceptibility gene testing - must be regarded with caution as few conclusions can be drawn from such examinations due to the complicated nature of the disorder. Presumably, pathogenesis is likely to be influenced by a myriad of genetic and acquired risk factors, which are likely to exert varying degrees of influence at different stages of life. In this dissertation, I present three research articles on the relative roles of given and acquired risk factors for cognitive impairment in aging. Specifically, the relative contribution of high levels of plasma total cholesterol and diabetes mellitus type II as acquired risk factors on

the one hand, and the role of the two common genetic risk variants of the *Apolipoprotein E* gene (*APOE*) and the *Catechol-O-Methyltransferase* (*COMT*) gene as given risk factors on the other hand, to cognitive impairment in aging were investigated. The data presented here are derived from the *Interdisciplinary Longitudinal Study of Adult Development and Ageing* (ILSE, Schönknecht, Pantel, Kruse & Schröder, 2005), a prospective longitudinal study that was initiated in 1992 and comprises two birth cohorts born between 1950 and 1952 (N=502) or 1930 and 1932 (N=500). The three articles "*Cholesterol in Mild Cognitive Impairment and Alzheimer's disease in a birth cohort over 14 years"* (Toro et al., 2014, **Paper 1**), "*The COMT p.Val158Met polymorphism and cognitive performance in adult development, healthy aging, and Mild Cognitive Impairment"* (Degen et al., 2015, **Paper 2**), and "*Diabetes Mellitus Type II and cognitive capacity in healthy aging and Mild Cognitive Impairment"* (Degen, Toro, Schönknecht, Sattler, & Schröder, 2016, **Paper 3**) are briefly summarized and placed into empirical contexts in Sections 2.1, 2.2, and 2.3. Afterwards, theoretical and practical implications of the respective results are discussed in Section 3.

2. Theoretical and Empirical Background

Besides age, as the most consistent risk factor for the development of MCI/AD, a multitude of genetic, psychosocial and environmental factors have been prescribed an important role in pathogenic processes. These factors are likely to interact and/or mediate each other, rendering a clear-cut classification into genetic, psychosocial and environmental factors unfeasible. These difficulties persist when attempting a strict delineation of genetic from acquired risk factors, given the plausible, albeit vaguely known hereditary influences on conditions acquired during a lifetime, as is the case in hypercholesterimia and diabetes mellitus type II. Hence, the risk factors referred to as “acquired” in this dissertation are potentially influenced by genetic predispositions and are very plausibly the result of complex gene*environment interactions. Nevertheless, the term is used to distinguish direct genetic influences from conditions that are more likely to originate in lifestyle factors, e.g. malnutrition.

Genetic association studies have substantiated the role of the *APOE* gene in the occurrence of sporadic AD, while the *amyloid precursor protein* (*APP*), the *psenelin 1* and *2* as well as the *sortilin-related receptor* genes are implicated in the development of familial dementia. In addition to these, the *COMT*, the *serotonin 2a receptor* (*5HT2a*), the *serotonin transporter* (*5HTTLPR*), the *brain derived neurotrophic factor* (*BDNF*), and the *glutamate receptor metabotropic* (*GRM3*) genes have been associated with general age-related decline of cognitive functioning (Kremen & Lyons, 2011). At the same time, cerebrovascular and lifestyle factors, including hypertension, high cholesterol levels, diabetes mellitus type II, heart disease, smoking, and obesity/malnutrition have been linked to the development of MCI/AD (Kivipelto et al., 2005; Luchsinger et al., 2005; van der Flier & Scheltens,

2005). However, none of these factors is sufficient or necessary for MCI/AD to occur illustrating the likely complexities of disease pathogenesis.

The intricate unfolding of given and acquired risk factors in cognitive decline is likely to vary as a function of age as inter-individual neural and cognitive variability increases. According to the *resource modulation hypothesis* (Lindenberger et al., 2008) losses in neurochemical and structural cerebral resources, such as age-related dopamine decline and atrophic changes, modulate the effects genes exert on cognitive functioning. Accordingly, genetic determinants exert increasing influence on cognition as we age. The hypothesis is based on the premise that heterogeneity in cognitive performance increases from early relative to late adulthood when resources are typically declining. However, with depleted resources – as is the case in AD – the influence of genes is hypothesized to dwindle (Lindenberger et al., 2008).

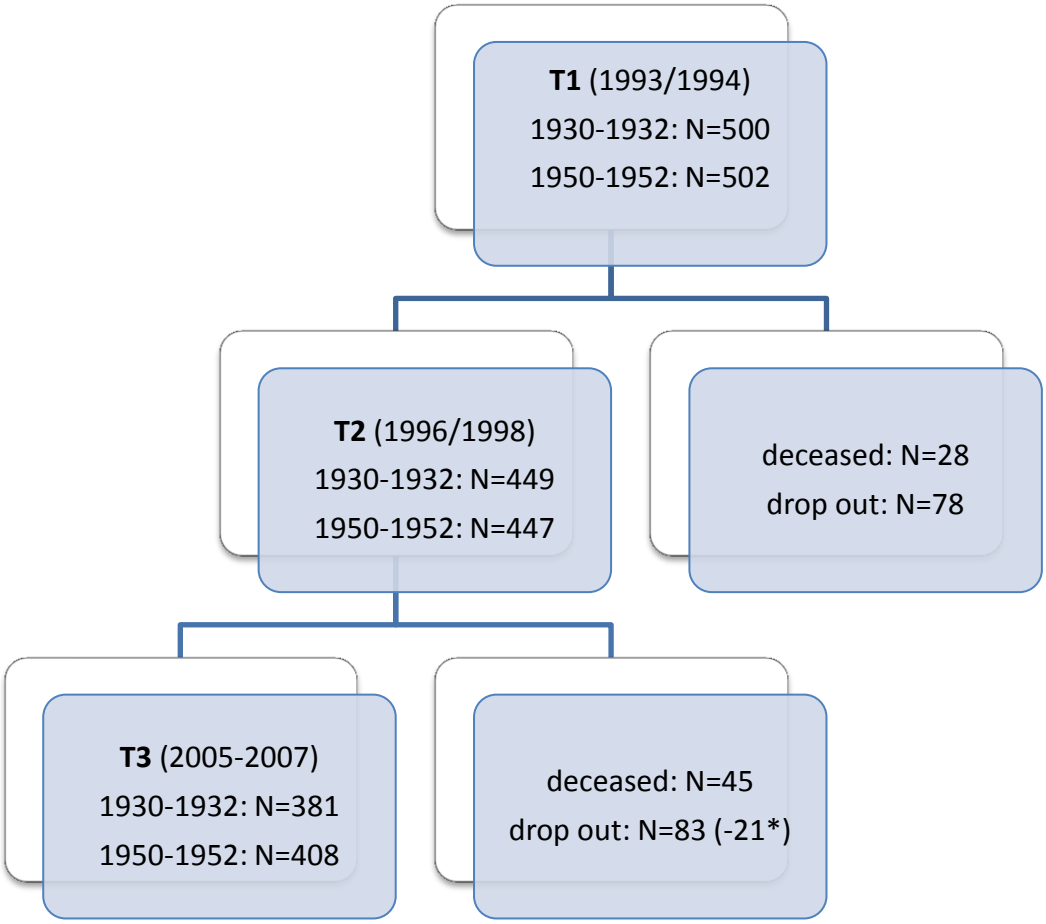
In clinical practice however, it is often observed that lifestyle factors such as the incorporation of regular physical and mental exercise may alter the trajectory of cognitive functioning in old age beneficially, suggesting that genetic dispositions become less pivotal during aging. The effects of learning on patterns of cerebral activation in the elderly corroborate this hypothesis as training may lead to reorganization of neural recruitment and more efficient processing. Thus, the aging brain remains a high degree of plasticity (Degen & Schröder, 2014). Related to these observations is the concept of *cognitive reserve*, which implicates that a given degree of neuropathological change can lead to differential degrees of functional impairment (Stern, 2002; Schröder & Pantel, 2011). As a result, some individuals with AD-related pathology are more resilient and may only display little functional impairment due to higher compensatory proficiency and functional plasticity (Schröder & Pantel, 2011; Sattler, Toro, Schönknecht, & Schröder, 2012). Cognitive reserve is typically operationalized via years of education, socio-economic status, chosen profession,

and lifestyle factors suggesting that these factors serve a compensatory role in AD-related functional impairment. Thus, the *resource modulation hypothesis* assumes an increase in the extent to which genetic vulnerability translates into functional impairment during aging, while studies on cognitive reserve and functional plasticity posit that age-related cognitive decline can, to a certain extent, be compensated for by environmental, i.e. lifestyle factors.

Scientifically, the course in which given and acquired factors (and combinations thereof) become apparent in a complex construct such as cognitive functioning throughout a lifespan can be most reliably assessed using large prospective longitudinal studies. The *Interdisciplinary Longitudinal Study of Adult Development and Ageing* (ILSE) is a population-based, prospective, longitudinal study that was initiated in 1992. Two birth cohorts born between 1930 and 1932 (N=500) or born between 1950 and 1952 (N=502) were examined at three examination waves in 1993/1994 (T1), between 1996 and 1998 (T2), and between 2005 and 2007 (T3). A fourth examination is currently being conducted and was initiated in 2014. At each examination wave, careful screening for physical and mental health was conducted by trained physicians. Psychiatric diagnoses were established using the German version of the Structured Clinical Interview for the Diagnostic and Statistical Manual Version III -Revised (DSM III-R; Wittchen et al., 1991) and the diagnostic criteria for aging-associated cognitive decline of the International Psychogeriatric Association working Party (Levy, 1994). To assess cognitive capacity, the subtests Word List and Digit Symbol Test of the Nuremberg Age Inventory (Oswald & Fleischmann, 1991), the subtests Mosaic Test, and Finding Similarities of the Hamburg Wechsler Intelligence Test Battery for adults (HAWIE-R; Tewes, 1991), the subtests Word Fluency and Visual Thinking of the Performance Evaluation System (“Leistungsprüfsystem”; Sturm, Willmes & Horn, 1993), as well as

the Attentiveness Endurance Test “D2” (Brickenkamp, 1978), were conducted among others. An overview of the participant flow between T1 and T3 can be seen in *Figure 1*. The study design allows for the investigation of various protective and risk factors in the course of healthy aging and the development of MCI/AD-related pathology over a period of 14 years (T1-T3).

Figure 1 Participant flow ILSE



***21 individuals participated in T3, but not in T2**

2.1. Cholesterol in Mild Cognitive Impairment and Alzheimer's disease in a birth cohort over 14 years (Paper 1)

Higher levels of total cholesterol in midlife have frequently been associated with an increased risk for developing dementia in later life (Solomon, Kivipelto, Wolozin, Zhou & Whitmer, 2009; Kivipelto & Solomon, 2006; Anstey, Lipnicki, & Low, 2008; Whitmer, Sidney, Selby, Johnston & Yaffe, 2005), while a gradual decrease of total cholesterol levels is observed before the onset of cognitive symptoms - possibly reflecting disease progression (Solomon et al., 2007; Mielke et al., 2010; Stewart, White, Xue, & Launer, 2007). Moreover, it has been suggested that the administration of cholesterol-lowering medication may serve a protective function (Dufouil et al., 2005; Wolozin et al., 2007; Li et al., 2007). High levels of total cholesterol have been linked to a modulation of the expression of the *APOE* and *APP* genes, thereby affecting β-amyloid secretion (Tokuda et al., 2000; Howland et al., 1998). *APOE* is a lipoprotein that is involved in the metabolism of cholesterol with a common variant of the *APOE* gene - the ε4 allele - displaying the least efficient cholesterol-clearing capacity relative to the two other variants ε2 and ε3 (Whalley, 2015). The *APOE* gene is an established susceptibility gene that has been reliably demonstrated to be a risk factor for decrements to cognitive functioning (Small, Rosnick, Fratiglioni, & Bäckman, 2004), the occurrence of sporadic AD as well as with earlier onset of familial AD (Whalley, 2015). A meta-analysis conducted on 77 studies (N=40.942 cognitively healthy adults) confirmed that the presence of ε4 alleles exerts adverse effects on cognitive functioning in healthy adults, particularly pertaining to episodic memory, global cognitive functioning, executive functioning and perceptual speed (Wisdom, Callahan & Hawkins, 2011). The authors found that people without the ε4 allele performed between .003 and .140 standard deviations

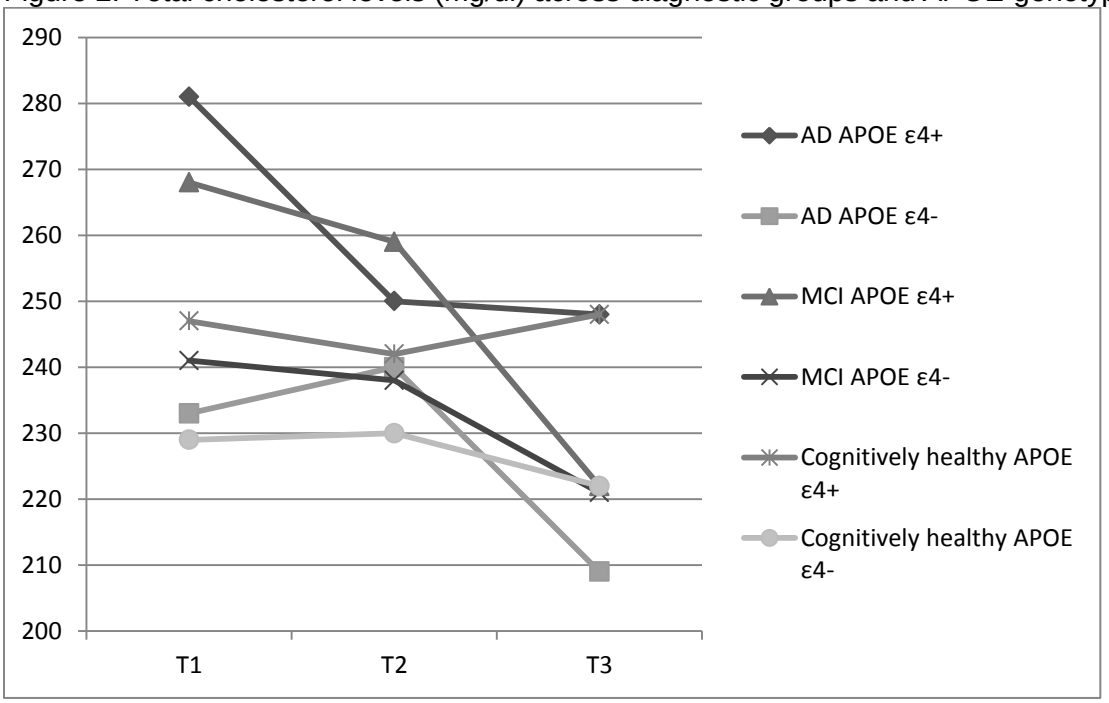
above those with the ε4 allele. Moreover, age resulted in larger effect size differences in tests assessing episodic memory and global cognitive ability between ε4 carriers and non-carriers, supporting the assumption that this particular genetic effect increases across the lifespan.

In the article "*Cholesterol in Mild Cognitive Impairment and Alzheimer's disease in a birth cohort over 14 years"* (Toro et al., 2014; **Paper 1**) our research group has looked at the role of plasma total cholesterol levels in the development of MCI/AD with the *APOE* genotype as a potential modulator across a 14-year interval (T1-T3). We hypothesized relatively higher levels of total cholesterol in midlife (T1) in individuals who developed MCI/AD at T3 in comparison to individuals who remained cognitively healthy. A total of 381 participants from the older birth cohort of the ILSE returned for T3. After exclusion of participants with other psychiatric disorders and those who refused *APOE* genotyping, 222 participants were included in the analyses. Total cholesterol was determined using Advia® 2400 Chemistry System from Siemens Healthcare Diagnostics, while genomic DNA was extracted from whole blood using the High Pure polymerase chain reaction (PCR) Template Preparation Kit.

Diagnostic groups (AD, n=22; MCI, n=82 and healthy controls, n=118) did not differ in terms of sex, body mass index, statin use, and *APOE* ε4 allele distribution, although a slightly higher proportion of ε4 carriers was observed in AD patients (27.3%) relative to MCI patients (22%) and healthy controls (23.7%). Interestingly, participants diagnosed with AD or MCI at T3 displayed higher levels of total cholesterol at T1 than healthy control participants with $F(2,221)$=3.179, p=0.044. Repeated measures analysis confirmed an interaction effect of time between examination waves and cognitive diagnoses, suggesting that total cholesterol levels declined between T1 and T3 for individuals diagnosed with MCI/AD, while remaining

stable for healthy controls $F(4,438)=3.88$, $p<.005$ (Figure 2). In addition, total cholesterol levels were higher in *APOE* ε4 carriers as indicated by a main effect of the *APOE* genotype $F(1,216)=14.39$, $p<.005$. We did not identify an interaction of time and *APOE* genotype suggesting that levels of total cholesterol did not change over time as a function of the *APOE* genotype.

Figure 2. Total cholesterol levels (mg/dl) across diagnostic groups and *APOE* genotypes



Our findings corroborate a bidirectional relationship between plasma total cholesterol and MCI/AD such that high total cholesterol in midlife is a risk factor for the development of MCI/AD while an observed decrease of total cholesterol may be associated with disease progression (Mielke et al., 2005; Stewart, White, Xue, & Launer, 2007). Thus, high levels of total cholesterol in midlife may serve as an early indicator for an increased risk to develop MCI/AD and bear implications for secondary preventive measures in this at-risk population. By drawing on one of the two birth cohorts of the ILSE, we were able to adjust for potential age effects. However, differences between cohorts in plasma total cholesterol are likely to exist, given that

lifestyle factors diverge between the cohorts. In particular, diets are likely to be different for the older cohort, born before World War II, than for the younger cohort. The younger cohort was not taken into consideration in this study due to the still low prevalence of MCI/AD in this population. Further research on levels of total cholesterol in young to middle adulthood and its predictive value for the occurrence of MCI/AD is of great importance to initiate disease prevention and adequate training approaches as early as possible.

No significant difference in the *APOE* ε4 proportion emerged for individuals diagnosed with MCI/AD and healthy controls in this particular sample contradicting findings from large epidemiological studies (National Institute on aging/Alzheimer's Association Working Group, 1996; Kivipelto, Helkala, & Laakso, 2002). The absence of a direct association between *APOE* ε4 frequency and diagnoses of MCI/AD in this sample may be due to a relatively low AD frequency at T3 in the older birth cohort. While systematic drop-out, with individuals suffering from severe concomitant conditions being less likely to participate, selection effects, due to the application of strict exclusion criteria (presence of other forms of dementia, Mild Cognitive Disorder, affective disorders, anxiety disorders, substance abuse disorders) and reliance on individuals who agreed to *APOE* genotyping, may have influenced the results, the return rate is generally regarded high in the older birth cohort with n = 381, 76.2% of the baseline sample after 14 years. Moreover, the prevalence of MCI/AD at T3 paralleled numbers reported in other population-based studies (Bischkopf, Busse & Angermeyer, 2002; Ferri et al., 2005), implying that selection effects are rather unlikely. As a result, we conclude that high levels of total cholesterol at age 60 with a subsequent decline in total cholesterol is indicative of the development of MCI/AD a decade later. This relationship is independent of the *APOE* genotype.

2.2. The COMT p.Val158Met polymorphism and cognitive performance in adult development, healthy aging, and Mild Cognitive Impairment (Paper 2)

COMT is an enzyme that is involved in the modulation and reduction of dopamine in the frontal cortex, where it inactivates neurotransmission (Mattay & Goldberg, 2004; Egan, Goldman & Weinberger, 2002). The *COMT* gene has a functional genetic polymorphism that results in the substitution of the amino acid Valine (*Val*) with Methionine (*Met*) allowing for three variants at codon 108/158: *Val/Val, Met/Met* and *Val/Met*. The *Val* allele is more temperature-resistant and its resultant enzyme more active. It is therefore associated with reduced dopamine levels and inferior cognitive performance, while the *Met* allele is, in turn, associated with reduced enzyme activity, higher levels of dopamine and superior cognitive performance (Savitz, Solms & Ramesar, 2006; Sheldrick et al., 2008).

The potential effect of *COMT* on cognition throughout the lifespan remains a subject of debate. A meta-analysis did not identify such an effect from cross-sectional data (Barnett, Scoriels, & Munafo, 2008), while results reported by Nagel et al. (2008) indicate that older (between 60 and 70 years of age) individuals carrying the *Val* allele displayed a higher number of perseverative errors in the Wisconsin Card Sorting Test than non-carriers. This discrepancy was absent for the younger participants (aged 20 to 30). The authors concluded that carriers of the *COMT Val* allele undergo greater loss in dopamine signaling than *Met* carriers during aging. However, this conclusion seems premature given the cross-sectional nature of the design chosen. In a longitudinal design de Frias et al. (2005) found that *Val/Val* carriers' performance on tasks of executive functioning declined over a five-year interval in contrast to *Met* carriers' performance. In particular, the researchers

identified a *COMT**age interaction for middle-aged adults at ages 50-60 years. Contrarily, Fiocco et al. (2010) found that individuals (aged 70-79) with a homozygous *Val/Val* genotype displayed significantly less decline in performance on the Digit Symbol Substitution Test than carriers of the *Met/Met* genotype across an eight-year interval indicating an approximation of genotypes in cognitive test performance in aging.

The article "*The COMT p.Val158Met polymorphism and cognitive performance in adult development, healthy aging, and Mild Cognitive Impairment"* (Degen et al., 2015; **Paper 2**) examines the role of the *COMT* genotype in cognitive functioning, following cognitively healthy participants from the younger birth cohort as well as cognitively healthy individuals and participants diagnosed with MCI from the older birth cohort of the ILSE over 14 years. We hypothesized that potential discrepancies between *Val* allele carriers and *Met* allele carriers of the *COMT p.Val159Met* polymorphism are particularly pronounced in cognitive tests assessing prefrontal cortex activity (i.e. executive functioning and mental flexibility) and would be more pronounced in the older birth cohort than the younger birth cohort, given relatively greater loss of striatal and extrastriatal dopamine. After exclusion of individuals with Mild Cognitive Disorder, AD, other forms of dementia, or affective disorders, 587 individuals (younger cohort: n=306; older cohort: n=281) were included in the analyses. DNA was extracted from whole blood using the Nucelon® Genomic DNA Extraction Kit BACC1. *COMT* genotype was determined as a restriction fragment length polymorphism after PCR amplification and digestion with NlaIII, as described by Lachman et al. (1996). Individual repeated measures analyses were conducted for the subtests Word List, Digit Symbol Test, Mosaic Test, Finding Similarities, Word Fluency and Visual Thinking as well as the Attentiveness Endurance Test "D2" from the neuropsychological test battery.

Analysis of Variance yielded no significant differences between genotypes with respect to demographic and clinical characteristics (age, education, APOE genotype, sex, cohort, cognitive diagnoses). However, significant differences between genotypes were identified for baseline cognitive performance, specifically in the subtests “D2” $F(2,580) = 3.40$, $p=.034$ and the Digit Symbol Test $F(2,584) = 3.77$, $p=.023$ such that individuals carrying the *Val/Val* genotype performed poorer than individuals carrying the *Met/Met* genotype or the heterogeneous genotype.

Results of a repeated measures analyses for healthy individuals from the older and the younger birth cohort illustrate a significant interaction of the *COMT* genotype and cohort for the Digit Symbol Test $F(2,451)=3.326$, $p=.037$ suggesting an effect of the *COMT* genotype for the younger, but not the older cohort (Figures 3 and 4). Also, a triple interaction between the *COMT* genotype, cohort and time emerged for the mosaic test with $F(2,254)=4.909$, $p=.008$ illustrating that trajectories in performance differed for carriers of different *COMT* genotypes from the two cohorts. Another repeated measures analysis was performed for the older birth cohort only, this time including individuals diagnosed with MCI. In this set of analyses, no significant main or interaction effect of the *COMT* genotype occurred.

Figure 3 Digit Symbol Test performance across *COMT* genotypes old cohort

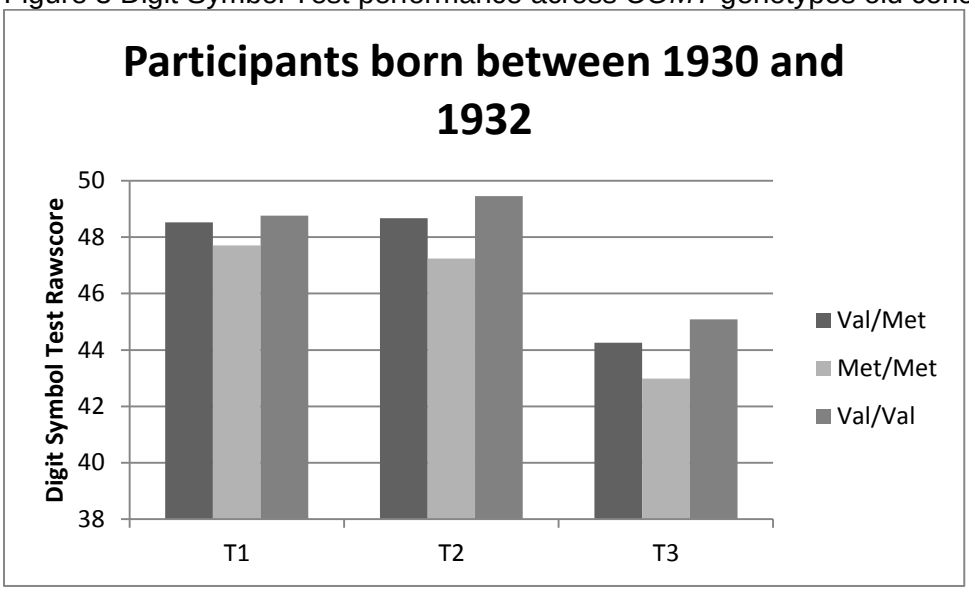
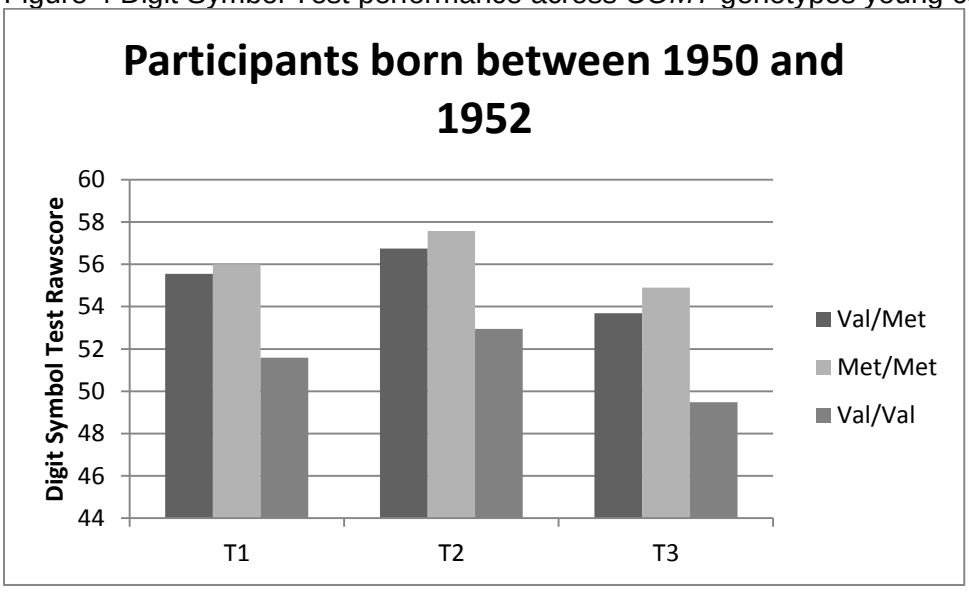


Figure 4 Digit Symbol Test performance across *COMT* genotypes young cohort



We find that, at baseline, *Val/Val* allele carriers perform significantly worse on tests of mental flexibility and attention than *Val/Met* or *Met/Met* allele carriers. The absence of an interaction effect between *COMT* and time (between examination waves) contradicts the idea of an approximation of different genotypes in the course of aging as demonstrated by Fiocco et al. (2010) but rather suggests a preservation of differences between genotypes in the younger cohort. Thus, our results imply

fundamental differences between birth cohorts that are independent of aging per se. Thus, no effects of the *COMT* genotype on cognitive performance in healthy aging and/or the occurrence of MCI were identified. Cohort differences may originate in lifestyle choices and/or environments, which very plausibly differ between individuals born 7-9 years before, and 5-7 years after World War II (nutrition, health care provision, social security, etc.). Interestingly, studies have identified neurological differences between carriers of different *COMT* genotypes (e.g. inefficient cortical processing) in the absence of behavioral differences (Dennis et al., 2010), implying that *Val/Val* carriers may be able to compensate reduced dopamine signaling in neuropsychological tests. The identification of neurological differences and specific factors that would lead to differential gene expression in cognitive functioning in the younger but not the older cohort is outside the scope of this article but augurs a variety of very interesting research ideas.

2.3. Diabetes mellitus type II and cognitive capacity in healthy aging and Mild Cognitive Impairment (Paper 3)

With an expected global increase of diabetes mellitus type II prevalence in people aged 65 and older (Wild, Roglic, Green, Sicree, & King, 2004) and a general understanding that this disease is associated with an increased risk for the development of dementia (for meta-analysis of prospective studies see Gudala, Bansal, Schifano, & Bhansil, 2013) diabetes mellitus type II is one of the most consistently described acquired risk factors for the development of MCI/AD. Cukierman, Gerstein and Williamson (2005) concluded that diabetes mellitus type II leads to a 1.2 to 1.5 fold greater decline in cognitive functioning and a 1.6 fold greater risk for developing dementia relative to healthy controls. The complex association between diabetes mellitus and dementia is assumed to be mediated by vascular factors. In a previous study, our research group was able to demonstrate that diabetes mellitus type II was associated with psychomotor slowing, but not with memory decline, which is typically compromised by AD pathogenesis (Toro, Schönknecht & Schröder, 2009). Contrarily, Awad, Gagnon, & Messier (2004) and Strachan, Deary, Ewing, & Frier (1997) find that diabetes mellitus-related deficits are consistently found with respect to verbal memory and processing speed, while other cognitive domains (visuospatial functioning, attention, language functioning) remain largely preserved.

In "*Diabetes Mellitus Type II and cognitive capacity in healthy aging and Mild Cognitive Impairment"* (Degen et al., 2016; **Paper 3**) we investigated the putative influence of diabetes mellitus type II and disease duration on cognitive functioning in healthy aging and the development of MCI/AD by drawing on the older birth cohort of the ILSE. To assess cognitive functioning, the subtests Word List, Digit Symbol Test,

Mosaic Test, Finding Similarities, Word Fluency and Visual Thinking, as well as the Attentiveness Endurance Test “D2” were considered. Individuals with Mild Cognitive Disorder and Vascular Dementia, as well as individuals diagnosed with affective or anxiety disorders were excluded. A distinction was made between individuals that had suffered from diabetes mellitus type II at T1 already and those that had not. We hypothesized that (1) diabetes mellitus type II is associated with decrements to tasks assessing psychomotor speed in healthy aging, (2) that diabetes mellitus type II aggravates cognitive decline in MCI/AD and (3) that disease duration is negatively associated with cognitive functioning.

At T3, there were 27 participants with both MCI/AD and diabetes mellitus type II, 108 participants with MCI/AD but without diabetes mellitus type II, and 26 individuals without MCI/AD but with diabetes mellitus type II and 134 participants without MCI/AD or diabetes mellitus type II. No difference in distribution of diabetes mellitus type II in the MCI/AD and healthy control groups was identified by Chi-Square test $\chi^2(1, n=295) = 0.699$, $p=.403$. However, the prevalence of diabetes mellitus type II in participants with MCI/AD was slightly higher (20.61%) than in those without a MCI/AD (16.25%). Repeated measures analyses yielded no significant main effects of diabetes mellitus type II on cognitive performance, but an interaction effect of time (between examination waves) and diabetes mellitus type II for the Digit Symbol Test $F(4,468) = 3.23$, $p=.012$ suggesting a steeper decline in cognitive performance for individuals diagnosed with diabetes mellitus type II at T1 and T3, relative to individuals diagnosed with diabetes mellitus type II at T3 only and those not suffering from diabetes mellitus type II (Figure 5). The same pattern was observed for the subtest visual thinking $F(4,466) = 2.76$, $p =.027$ (Figure 6). Moreover, a triple interaction of time (between examination waves), diabetes mellitus type II and diagnosis of MCI/AD was observed for the subtest visual thinking $F(4,464)$

= 2.81, *p* =.025. Accordingly, individuals with both MCI/AD and diabetes mellitus type II exhibited a steeper decline between T1 and T3 than other individuals. Additionally, performance of individuals with MCI/AD and diabetes mellitus type II diagnosis at T3 (but not T1) exhibited a more pronounced decline between T1 and T2 than those with MCI/AD and no diabetes mellitus type II.

Figure 5 Trajectories for performance on the Digit Symbol Test across diabetes mellitus type II diagnostic groups

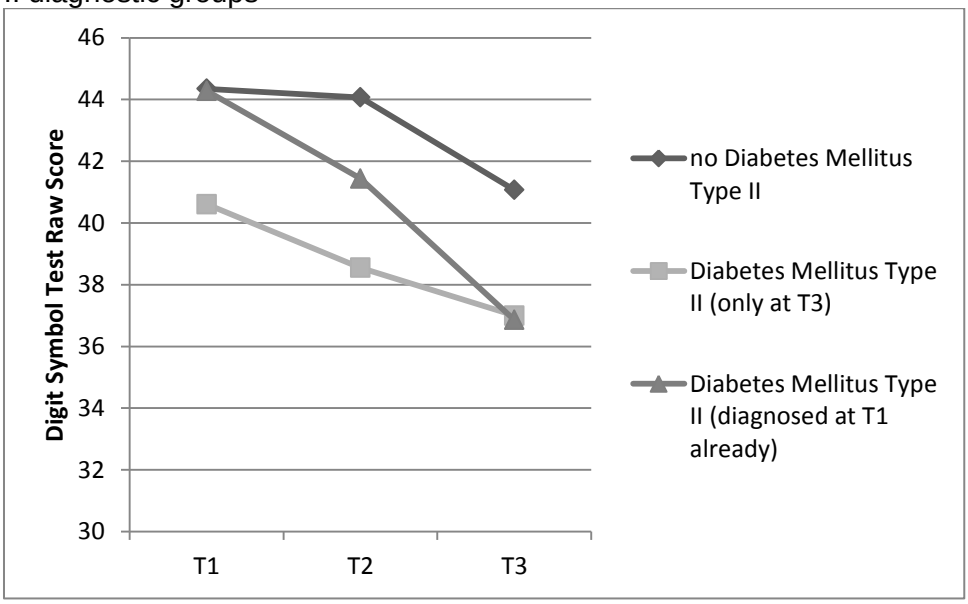
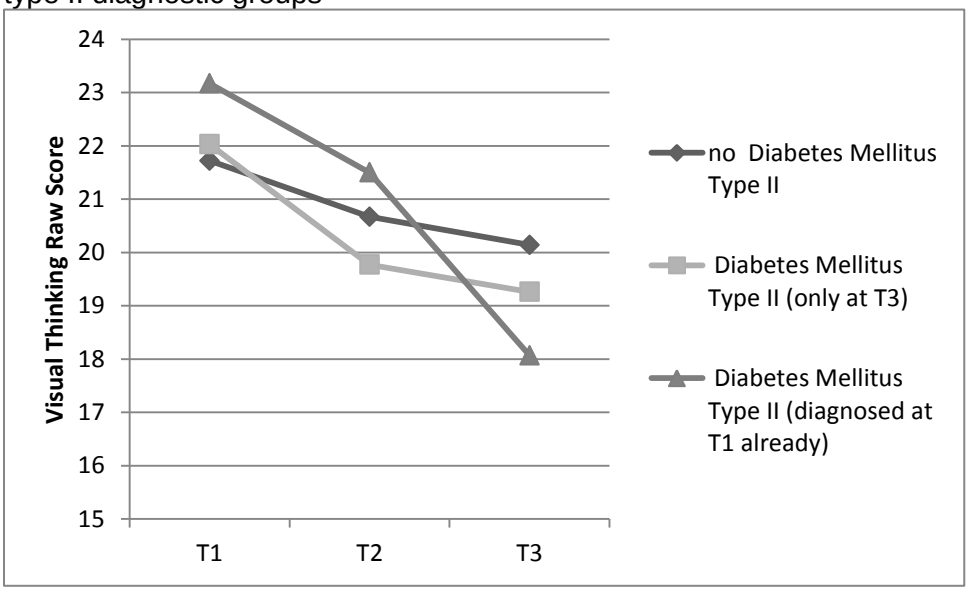


Figure 6 Trajectories for performance on the Visual Thinking subtest across diabetes mellitus type II diagnostic groups



We found support for the assumption that diabetes mellitus type II may aggravate the course of cognitive decline in aging after the disease has been present for a longer period of time. Thus, it is plausible that diabetes-related cognitive changes progress slowly and behavioral and neurophysiological manifestations become apparent somewhat later with disease progression. This effect mostly pertains to tasks involving psychomotor speed and executive functioning. Both findings are in line with previous studies reporting that memory-related cognitive domains remain largely preserved, while psychomotor speed and executive functioning are affected by diabetes mellitus type II (Toro, Schönknecht & Schröder, 2009) and the respective effects are evident only at later stages of the disease (van den Berg, 2010). Prolonged exposure to diabetes mellitus type II may very plausibly lead to neurophysiological changes, which in turn results in psychomotor slowing. Our findings contradict results from cross-sectional studies, according to which effect sizes consistently range between -.3 and -.6 on cognitive tests (Awad, Gagnon, & Messier, 2004). The absence of significant associations between diabetes mellitus type II and the diagnosis of MCI/AD maybe partially attributed to selective dropout, given that 50% of individuals with diabetes mellitus type II at T1 did not follow up to T3 (n=22). However, prevalence of MCI/AD in this sample resembles rates reported in other population-based studies, rendering selective dropout of patients suffering from MCI/AD less likely. It is reasonable to assume that diabetes mellitus type II may serve as a model for other concomitant diseases (e.g. hypertension) that, if present for a prolonged period of time, may cause neurophysiological damage, such as periventricular hyperintensities (van Harten et al., 2007), white matter lesions and cortical and subcortical atrophy (Manschot et al., 2006). These cerebrovascular changes are likely to affect cognitive functioning in the long term. Early diagnoses and effective disease management can therefore bear important implications for the

preservation of cognitive functioning in old age. As a result, diabetes mellitus type II may be illustrative for other concomitant diseases (e.g. hypertension), which conceivably lead to neurophysiological changes and may, after prolonged exposure, reinforce cognitive decline in aging.

3. Discussion

The investigation of the relative contribution of given and acquired risk factors for the development of MCI/AD across the lifetime bears important implications for the identification of at-risk populations, the implementation of preventive measures as well as effective disease management. Not surprisingly, patient requests for information are on the rise as reflected in the emergence of genetic counseling units, and ready availability of gene susceptibility test procedures. However, such procedures must be interpreted with caution. As our results illustrate, genetic predispositions adopt subsidiary roles in age-related cognitive decline and the development of MCI/sporadic AD, relative to environmental and/or lifestyle factors.

Our results are in line with the clinical concept, according to which efforts to improve concomitant conditions such as diabetes mellitus type II, hypercholesterimia, or hypertension are warranted to relieve cognitive dysfunction during aging. While this is frequently observed in clinical practice, only a handful of studies provide empirical support. In this respect, the protective function of statin use is considered most established (Dufouil et al., 2005; Wolozin et al., 2007; Li et al., 2007). Thus, preventive measures targeting high levels of total cholesterol in midlife, but also diabetes mellitus type II may very plausibly alter and/or stabilize cognitive functioning in old age by preventing micro-vascular damage that may result from prolonged exposure to these conditions. Thus, diagnoses of diabetes mellitus type II or hypercholesterimia in midlife may help to identify individuals that are at risk for developing MCI/AD or Mild Cognitive Disorder. The promotion of healthy lifestyles in midlife, including proper nutrition, is of particular importance to these patient groups.

While prolonged exposure to concomitant diseases influences cognitive aging very conceivably, our results imply that testing for genetic risk factors is of limited

prognostic value in establishing individuals' probabilistic risks for the development of MCI/AD. While having a relative with AD may increase the probabilistic risk of developing AD – given the presence of certain susceptibility genes -, research examining the relative contributions of genes and environmental factors in developing sporadic AD illustrate the relatively greater importance of environmental factors. These factors may include not only concomitant diseases, but also exposure to viruses and bacteria, diet, vitamin deficiencies or nutrients, history of head trauma, exposure to toxins and pollutants, as well as lifestyle factors (e.g. physical and cognitive activity, education).

Somewhat surprisingly, no statistically significant association between the *APOE* genotype, a well-established susceptibility gene, and the development of MCI/AD was identified in our sample. Some studies suggest that the presence of ε4 alleles influences the age of onset at which AD occurs, but does not confer information about the overall lifetime risk for AD (Khachaturian, Corcoran, Mayer, Zandi, & Breitner, 2004; Meyer et al., 1998). Other studies suggest that the presence of a ε2 allele resembles a protective factor against AD (Corder et al., 1994), which has not been taken into account here, due to the relatively low prevalence of the ε2 allele in our immediate geographical region (Corbo & Scacchi, 1999). For the *COMT* polymorphism, a weak association was identified with performance in tests assessing mental flexibility, but not with the development of MCI/AD. This association was limited to the younger cohort of the ILSE contradicting assumptions based on the *resource modulation hypothesis acc*ording to which genetic influences increase across the lifetime. Our results suggest that the link between genetic predispositions and the occurrence of sporadic AD/MCI remains weak at best. The use of genetic counseling (e.g. gene susceptibility testing) in sporadic AD must therefore be regarded with utmost caution. While individuals, whose parents or siblings are

affected by AD, may desire disclosure to be able to prepare adequately, arrange health care plans, or modify lifestyle factors, it is important to highlight the limitations of *APOE* susceptibility testing at this point. There is no clear genotype-phenotype association as genotypes may have variable expressions within families. As a result, *APOE* susceptibility testing lacks sensitivity and specificity, while the current lack of preventive options and likely difficulties in conveying probabilistic risk to patients may plausibly cause (additional) psychological harm to patients (Mayeux et al., 1998, Goldman et al., 2011).

Our results suggest that cohorts differ fundamentally, as the *COMT* polymorphism affects cognitive functioning in the younger, but not in the older birth cohort. This may be attributed to higher levels of cognitive reserve in individuals born between 1950 and 1952 as opposed to individuals born between 1930 and 1932. As a result, the young cohort displays higher compensatory proficiency, presumably due to lifestyle factors, including overall healthier lifestyles and medical supply, but also chosen profession and level of education. Notably, the two cohorts differ with respect to years of formal education (younger cohort: 14.04 ± 2.52; older cohort: 12.88 ± 2.80) with $t(1000) = -6.88$, $p<.0001$. In addition, differences between cohorts with respect to neuropsychological functioning have been described previously (Flynn, 1987). The so called “Flynn effect” describes a gradual increase of test scores on standardized intelligence quotient (IQ) tests between generations. Salthouse (1991) referred to the conservation of known birth cohort differences in overall cognition as *preserved differentiation.* As a result, younger cohorts may perform better on certain tests of cognitive functioning than older cohorts, while the degree of age-associated cognitive decline remains the same. *Differential preservation* on the other hand, assumes differential trajectories between birth cohorts. Finkel, Reynolds, McArdle, & Pedersen (2007) applied growth curve models to data from 806 participants from the

Swedish Adoption/Twin Study of Aging. Taking into consideration five examination waves across a 16-year interval and splitting the sample into two separate cohorts (born between 1900 and 1925 or 1926 and 1948) the authors identified significant differences between cohorts at age 67.5 years for verbal, spatial and memory abilities, but not for processing speed. These results suggest that younger birth cohorts' cognitive performance is superior to older birth cohorts' cognitive performance. In this light, we performed a preliminary analysis on differences in neuropsychological functioning and intelligence between the younger and the older cohort from the ILSE. While 447 individuals from the older birth cohort (mean age 66.41 +/- 0.97) completed the second examination wave in 1996/1998, a total of 187 individuals from the younger birth cohort have already completed the fourth examination wave that was initiated in 2014 (mean age 63.37 +/- 0.54). Analyses of Variance included education as a covariate and suggest significant differences between cohorts with respect to general intelligence, visuo-spatial thinking, attention, learning, and memory with the younger cohort outperforming the older cohort (see *Table 1*). Thus, differences between the two cohorts exist, potentially modifying the extent to which genetic and acquired risk factors influence cognitive functioning.

Table 1. Differences in neuropsychological functioning between cohorts of the ILSE

	Old birth cohort (1930/1932)	Young birth cohort (1950/1952)	ANCOVA (F, p)
Intelligence[1]	76.34 (±18.57)	87.90 (±20.92)	16.29, <.0001
Visuo-spatial thinking	20.48 (±6.73)	23.96 (±7.41)	11.80, .0006
D2 concentration	142.54 (±39.86)	143.30 (±44.90)	6.02, .0144
Verbal memory	5.29 (±1.48)	5.91 (±1.48)	10.28, .0014
Verbal Fluency	30.05 (±9.43)	31.17 (±9.91)	0.46, .4962

[1]=sumscore of general knowledge, finding similarities, complementing pictures, and mosaic test of the Wechsler Intelligence Test battery

Currently, the fourth examination wave of the ILSE is about to be completed. We expect a rise in MCI/AD prevalence in the older and the younger cohort, as age is the most consistently found risk factor for pathogenesis. Recent studies suggest that the prevalence of dementia is declining in spite of a demographic shift towards an aging society (Satizabal et al., 2016). However, while we are now able to directly compare the two birth cohorts at the same age, a comparison of individual trajectories across time will be visible only in future examination waves. According to *cognitive reserve* theory, individuals with higher levels of compensatory proficiency at their disposal display more rapid cognitive decline once AD pathology is evident. Specifically, individuals with higher cognitive reserve can compensate higher degrees of AD pathology until the “point of inflection” (Stern, 2009; p. 2018) is reached. Following this line of reasoning, clinical symptoms will occur at later stages of AD pathology, but the subsequent decline in cognitive functioning will be steeper for individuals with high cognitive reserve as opposed to low cognitive reserve (Stern, 2009). If cohort differences in the ILSE are attributable to different levels of cognitive reserve the observed cohort differences will remain stable at first, but a rapid approximation of cognitive trajectories is to be expected at later stages. Alternatively, differences between cohorts will remain stable, as illustrated by the concept of *preserved differentiation* (Salthouse, 1991).

It is for this reason that the continuation of the ILSE as a comprehensive and sustainable prospective research framework with a follow-up interval of 23 years is of utmost significance. A major strength of the study lies in the comparison between repeated measures over a long period of time. With a high return rate at T3 (78.74% of the baseline sample) and an expected return rate of approximately 550 (>50.00%) at T4 across a 23-year interval confirmatory analyses can be conducted in spite of expected drop-out. Also, future research may potentially target the inclusion of a new

birth cohort at ages 41-43 to systematically address differences between cohorts. From this, potential intervention studies could arise, targeting hypercholesterimia and diabetes mellitus type II, besides other important risk factors associated with MCI/AD. The potentially protective role of cholesterol-lowering medication may be of particular importance in this context. In addition, the identification of genetic risk factors could be enhanced by incorporating novel techniques, including neuroimaging and whole genome analyses rather than single nucleotide polymorphisms.

Cognitive performance in aging is modulated by a myriad of variables of different types. A clear-cut delineation of aging effects per se from effects of time, genes, or secular events is restricted in this and other observational settings. With a follow-up interval of 23 years (fourth examination wave 2014-2016) the ILSE represents a unique prospective research framework for the investigation of various determinants of healthy and pathological aging. However, when interpreting the results presented in this dissertation, some limitations in the respective analyses need to be considered. First, selective dropout may have influenced our results. For example, we found that 50% of individuals that were initially diagnosed with diabetes mellitus type II did not follow up to T3. Mielke et al. (2010) found that differences in levels of total cholesterol reached statistical significance only in the survivor analysis, and no significant differences appeared between the groups in the whole sample analysis. Given that we identified an effect of diabetes mellitus type II on cognitive functioning if it had been present at T1 already, we can assume that effects would be more pronounced, potentially expanding other cognitive domains if the entire sample was taken into consideration. Moreover, we expect that analyses regarding sources of attrition (death, moving away, illness, not traced, non-compliance) will yield no differences from other longitudinal studies.

The second limitation concerns a general drawback of genetic association studies and their reliance on a single genotype – phenotype association. A construct such as cognitive functioning in aging is the result of various genetic and environmental factors. In this light, different candidate genes affecting dopamine regulation and amyloid secretion are likely to be important and interact. The golden standard in identifying genetic risk factors is to look at unique features between individuals that develop AD and those that do not. However, while other diseases have certain biomarkers that reliably predict disease outcome, as is the case in cancer or HIV, this is less clear-cut in patients potentially affected by AD rendering definite conclusions of potential genotype-phenotype associations more difficult.

Likewise, MCI generally describes cognitive profiles that are marked by verifiable cognitive deficits that do not reach the severity typically observed in manifest dementia. Nevertheless, MCI is regarded a major risk factor for the development of dementia - Alzheimer's dementia (AD) in particular. Various conceptualizations of this particular "transition stage" exist and are commonly used in research. As such, a*ging-associated Cognitive Decline* as postulated by Levy in 1994 is defined by the presence of subjective complaints of cognitive decline (by the patient or a close relative), a progressive decline of cognitive functioning, and neuropsychological deficits in one of the following cognitive domains: memory and learning, attention and concentrations, language, visuo-spatial functioning and abstract thinking. A cognitive deficit is thereby any performance that is at least one standard deviation below age- and education-adjusted normative values. The reliance on neuropsychological test batteries in diagnosing can potentially be regarded as a limitation as these are largely classified by their content and can only partially reflect differential cognitive domains or phenotypes (review Harris & Deary, 2011). However, by using the same test battery throughout all examination waves

(and therefore focusing on the same cognitive constructs throughout) and relying on thorough and individual medical assessment by specialized physicians, diagnostic accuracy can be regarded as high in this study design.

4. Conclusion

The identification and characterization of given and acquired risk factors in pathological aging, i.e. the development of MCI/AD, is crucial for the development of preventive measures, the implementation of adequate interventions as well as effective disease management. We investigated the relative contribution of the well-established susceptibility genes *APOE* and *COMT* on the one hand, and diabetes mellitus type II and high levels of total cholesterol as acquired risk factors, on the other hand. Our results indicate that the susceptibility genes *APOE* and *COMT* can generally be regarded as subsidiary in evaluating one's probabilistic risk of developing cognitive dysfunction, MCI, or sporadic AD, while highlighting the importance of effectively treating concomitant diseases in midlife. Specifically, individuals suffering from high levels of total cholesterol or diabetes mellitus type II in midlife are at risk of cognitive dysfunction in old age.

Cognitive functioning in old age may benefit not only from effective treatment of the respective concomitant diseases, but the patient groups may be particularly susceptive to physical and cognitive training interventions. Likewise, given the protective function of *cognitive reserve*, the incorporation of educational measures and cognitively demanding recreational activities in daily routines is likely to promote healthy cognitive aging. The identification of differences between the two birth cohorts substantiates the important role of environmental/secular factors, including levels of education and medical provision, which could plausibly influence the extent to which genes influence cognitive functioning in old age.

Our findings bear important directions for future research, including the systematic investigation of cohort differences. Moreover, intervention studies specifically targeting frequently occurring concomitant diseases, including the

monitoring of neurophysiological correlates in a longitudinal setting are desirable and of great value to understanding the development of cognitive functioning during aging as well as the onset of pathological processes as typically observed in MCI/AD.

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Abbreviations

5HT2a = serotonin 2a receptor gene

5HTTLPR = serotonin transporter gene

AD = Alzheimer's disease

APOE = Apolipoprotein E

APP= Amyloid Precursor Protein

BDNF = Brain derived neurotrophic factor

COMT = Catechol-O-Methyltransferase

DNA = Deoxyribonucleic acid

DSM-III-R = Diagnostic and Statistical Manual Version III Revised

GRM3 = glutamate receptor metabotropic gene

HAWIE-R = Hamburg Wechsler Intelligence Test Battery Revised

IQ = intelligence quotient

ILSE = Interdisciplinary longitudinal study of adult age

MCI = Mild Cognitive Impairment

Met = Methionine

PCR = polymerase chain reaction

T1 = first examination wave of the ILSE

T2 = second examination wave of the ILSE

T3 = third examination wave of the ILSE

T4 = fourth examination wave of the ILSE

Val = Valine

Erklärung gemäß § 8 Abs. 1 Buchst. b) und c) der Promotionsordnung der Fakultät für Verhaltens- und Empirische Kulturwissenschaften

Promotionsausschuss der Fakultät für Verhaltens- und Empirische Kulturwissenschaften
der Ruprecht-Karls-Universität Heidelberg
Doctoral Committee of the Faculty of Behavioural and Cultural Studies, **of Heidelberg University**

Erklärung gemäß § 8 Abs. 1 Buchst. b) der Promotionsordnung der Universität Heidelberg
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ORIGINAL PAPER

Cholesterol in mild cognitive impairment and Alzheimer's disease in a birth cohort over 14 years

P. Toro · Ch. Degen · M. Pierer · D. Gustafson · J. Schröder · P. Schönknecht

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Abstract Animal epidemiological and clinical studies suggest that cholesterol is a risk factor for Alzheimer's disease (AD). Nevertheless, the relation of cholesterol to mild cognitive impairment (MCI), influence of APOE genotype and its changes in lifespan is controversial. We investigated the potential impact of plasma total cholesterol (TC) on development of MCI and AD in the interdisciplinary longitudinal study on adult development and aging, a representative birth cohort (born 1930–1932), examined in 1993/1994 (VT1), 1997/1998 (VT2), and 2005/2007 (VT3). Of 500 participants at baseline, 381 survived and were examined at VT3. After exclusion of participants with lifetime prevalence of major psychiatric diseases or mild cognitive disorder due to a medical condition, 222 participants were included in the analysis. At VT3, 82 participants had MCI, 22 participants had AD, and 118 were in good health. Participants with MCI and AD at VT3 evidenced higher TC levels at VT1 than those who were healthy. Higher TC levels at baseline were associated with an increased risk for cognitive disorders at VT3 (highest vs. lowest quartile: OR 2.64, 95 % CI 1.12–6.23, $p < 0.05$). Over the 14 year follow-up, TC levels declined in those with MCI and AD, but remained stable in those who remained healthy. These findings were not modified by APOE genotype or use of cholesterol-lowering medications. Our findings demonstrate that higher TC levels are observed long before the clinical manifestation of MCI and AD in patients without psychiatric or somatic comorbidities and are independent of APOE genotype.

Keywords AACD · AD · APOE · Cholesterol · ILSE · MCI

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Introduction

Cholesterol is considered to be involved in the pathogenesis of mild cognitive impairment (MCI) and Alzheimer's disease (AD). In view of that, clinical studies have demonstrated increased levels of cerebrospinal fluid 24-hydroxycholesterol in AD patients compared with healthy controls [1]. Likewise, epidemiological studies have established that higher total cholesterol (TC) in midlife is a risk factor for the development of AD [2–4] (for a review see [5, 6]), and that cholesterol-lowering agents, such as statins, may have a protective function [7, 8]. Interestingly, there is evidence that TC values decrease

more rapidly in subjects experiencing subsequent cognitive decline or dementia compared with individuals who remain healthy. Declining cholesterol levels appear to accompany aging specifically in AD and are probably a manifestation of underlying dementia-related neuropathology [9, 10].

Experiments on cell cultures provide a feasible explanation for this association, indicating that the accumulation of cholesterol in neurons results in an accelerated cleavage of amyloid precursor proteins into amyloidogenic components [1]. This may lead to the formation of amyloid plaques in susceptible brain regions with consecutive neuronal degeneration. Thus, experiments on hypercholesterolemic rabbits [2] find an association with blood cholesterol and enhanced amyloid deposition in the brain. Additionally, cholesterol deposits in amyloid plaques may promote the stability thereof.

Most of the prospective studies reporting on cholesterol and cognitive impairment or dementia [2–4] have involved northern European populations, which are generally characterized by a high prevalence of the APOEε4 allele. This is of particular importance, since the presence of the APOEε4 allele confers an increased risk of AD. The APOE protein is involved in Aß metabolism, and the affinity of APOE for Aß is increased in the presence of lipids [11]. Animal studies demonstrate that a high cholesterol diet affects not only TC plasma levels but also modulates APOE expression, and APP and Aß secretion [12].

In the present study, we investigated the role of TC levels in the development of MCI and AD, as well as the potential impact of APOE genotype. Specifically, a German population-based sample born between 1930 and 1932 drawn from the interdisciplinary longitudinal study on adult development and aging (ILSE) was considered in the course of three examination waves—extending over 14 years.

Materials and methods

Participants

The ILSE is a prospective study of adult development in Germany based on two birth cohorts born in 1930–1932 and 1950–1952 [13, 14]. At baseline, in 1993–1995, participants were randomly selected and recruited from the community registers in the urban regions of Leipzig (Saxony) and Heidelberg/Mannheim (Palatine), for which inclusion is mandatory for citizens aged 16 years and older in Germany. This recruitment procedure yielded a representative sample of the communities included [15]. Participants were subsequently recontacted over a 14 year period, in 1997–2000 and 2005–2008.

This study includes only those participants born 1930–1932 and who completed the 2005–2008 examination ($n = 381$). A description of this sample is as follows (see Fig. 1). At baseline, in 1993–1995 (VT1), 500 participants were examined. In the second examination (VT2) in 1997–1999, 449 persons were re-examined; and in 2005–2008 (VT3), 381 [76.2 % of the baseline sample, average age 74.3 (SD = 1.2) years] persons were examined. Of the 119 non-participants at VT3, 64 (53.8 %) had died, seven (5.9 %) no longer lived in the region, 39 (32.8 %) were ineligible, and nine (7.5 %) did not wish to participate.

Since MCI and AD were the primary outcomes of interest, additional exclusion criteria were applied at VT3, such that those who met criteria for other mental disorders such as vascular dementia, major depression, anxiety disorders, or mild cognitive disorder (MCI due to a medical condition as defined by ICD-10) were excluded. Thus, only those participants surviving from VT3—developing MCI or AD or remaining cognitively healthy—were included.

Given study exclusion criteria, the final sample at VT3 included 109 participants with MCI, 26 with AD, and 157 cognitively healthy individuals, without any psychiatric disorder. Of these, 70 participants refused APOE genotyping (39 without any psychiatric disorder, 27 with MCI and four with AD), leaving a final sample of 222 participants. Amount of missing data did not differ between diagnostic groups [$\chi^2(2) = 1.16$, $p = 0.56$].

The study was approved by the Ethical Committee of the University of Heidelberg. After a complete description of the study to the participants, written informed consent was obtained.

Psychiatric diagnoses at VT3

Psychiatric disorders were diagnosed using the German version of the Structured Clinical Interview for the DSM-III-R [16]. MCI was diagnosed according to the aging-associated cognitive decline criteria (AACD, International Psychogeriatric Association working Party, [17]) including (1) subjective impairment: A report by the individual or an informant that cognitive function has declined and (2) objective impairment: difficulties in any of the following cognitive domains, as indicated by neuropsychological test performance of at least one standard deviation below normal age and educational levels: memory and learning, attention and concentration, abstract thinking (problem solving, abstraction), language, and visuospatial functioning. Moreover, MCI patients were classified as amnestic or non-amnestic types, depending on the affected cognitive domain. AD and vascular dementia were diagnosed using the NINCDS–ADRDA and the NINDS–AIREN criteria, respectively [18, 19]. Additional methodological details have been described elsewhere [13, 14]. Particular care was taken to exclude participants with mild cognitive disorder

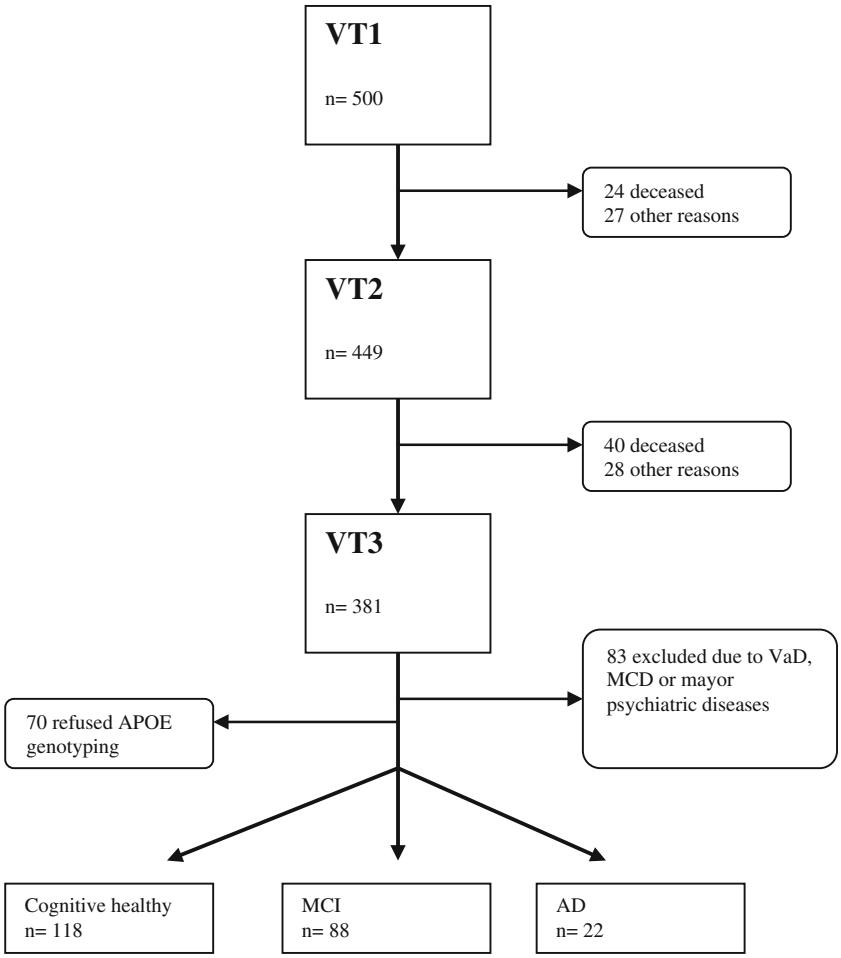

Fig. 1 Study design. *MCI* mild cognitive impairment, *MCD* mild cognitive disorder due to medical condition, *VaD* vascular dementia

(ICD-10), and major psychiatric disorders such as depression or substance abuse, since symptoms of these conditions overlap with dementia and other cognitive disorders. Clinical diagnoses were established by consensus of two psychiatrists (P.T., P.S.) under supervision of a specialist (J.S.) in geriatric psychiatry.

Laboratory measures

TC was analyzed with Advia® 2400 Chemistry System from Siemens Healthcare Diagnostics. Genomic DNA was extracted from whole blood using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer's instructions. APOE genotype was assessed using the LightCycler technology [20].

Survey measures

Participants were carefully screened for physical and mental health via questionnaires, extensive personal interviews, as well as medical and neuropsychological examinations at all visits (VT1, VT2, and VT3). The cognitive assessment included the mini-mental state examination (MMSE [21]), subtests of the Nürnberger–Alters–Inventar (NAI) [22] and the Leistungsprüfsystem [23], both of which are well-established and commonly used test batteries in Germany (for more details see [14]):

1. Memory and learning: immediate word list recall and delayed word list recognition (NAI)
2. Attention and concentration: Aufmerksamkeits–Belastungs-Test (d2 test [24].
3. Abstract thinking: similarities subtest (Hamburg–Wchsler–Intelligneztest für Erwachsene) [25].
4. Language-subtest of verbal fluency (Leistungsprüfungssystem).
5. Visuospatioal functioning: subtest of visual imagination (Leistungsprüfungssystem).

Statistics

Diagnostic groups were compared using analyses of variance with repeated measures for time. Post hoc Tukey's tests and χ^2 tests were used where appropriate. To address potential effects of the APOE genotype, groups were

additionally dichotomized according to the presence or absence of at least one e4 allele.

In order to assess the risk of MCI and AD associated with plasma TC, participants were divided into TC quartiles and odds ratios (OR) were calculated. Logistic regression analyses were performed to determine statistical significance at 95 % confidence intervals. In order to adjust for important potentially confounding variables, education, APOE genotype, socio-economic status, and gender were included into the logistic regression model.

SAS software (version 9.01; SAS Institute, Cary, NC, USA) was used for all statistical analyses.

Results

Demographic and clinical characteristics of the diagnostic groups are summarized in Table 1.

Diagnostic groups (AD, MCI, healthy controls) did not differ on the basis of sex, statin use, and APOEε4 allele. Moreover, no significant differences between diagnostic groups emerged with respect to body mass index (BMI) for VT1 ($F = 1{,}182$; n.s.), VT2 ($F = 1{,}786$; n.s.), and VT3 ($F = 1{,}226$; n.s.), smoking behavior for VT1 ($\chi^2 = 1.090$, $df = 2$, n.s.), VT2 ($\chi^2 = 0.276$, $df = 2$, n.s.), and VT3 ($\chi^2 = 0.224$, $df = 2$, n.s.), hypertension at VT1 (Fisher's exact test: n.s.), VT2 (Fisher's exact test: n.s.) or diabetes mellitus for VT1 ($\chi^2 = 0.289$, n.s.) and VT2 (Fisher's exact test: n.s.). AD cases were slightly older than the cognitively healthy. Length of formal school education was shorter in those with AD or MCI ($F = 13.23$, $p < 0.0001$). Mean MMSE scores differed between groups ($F = 100.10$, $p < 0.0001$) with the MCI group ranking in between participants with AD and the cognitively healthy. 19.5 % of MCI cases were classified as amnestic (scoring >1SD below average on NAI word list), 65.9 % were classified as non-amnestic.

TC levels at baseline differed between diagnostic groups with MCI and AD patients having higher TC levels than controls ($F = 3.179$, $p = 0.044$). Post hoc analysis revealed a significant difference between MCI and healthy controls ($p = 0.046$). Average TC levels declined during follow-up in MCI and AD, but were almost stable in participants who remained cognitively healthy at VT3. These findings were confirmed by a repeated measures ANOVA that yielded a main effect of time ($F = 15.51$, $df = 2/438$, $p < 0.0001$) and an interaction effect of diagnosis by time ($F = 3.88$, $df = 4/438$, $p < 0.005$), while diagnosis alone was not informative ($F = 0.90$, $df = 2/219$, $p = 0.4$, n.s.).

Diagnostic groups were also dichotomized according to presence of an APOEε4 allele (Table 2; also see Fig. 2). TC levels were significantly higher among those with any e4 allele ($p < 0.05$). A repeated measures ANOVA with time as within-subject factor revealed significant main effects for APOE and time, as well as a significant time by diagnosis interaction ($F = 14.39$, $df = 1/216$, $p < 0.005$; $F = 13.68$, $df = 2/432$, $p < 0.001$; and $F = 5.40$, $df = 4/432$, $p < 0.005$, respectively). No other main or interaction effect emerged (diagnosis: $F = 0.77$, $df = 2/216$; diagnosis × APOE: $F = 0.50$, $df = 2/216$; time × APOE: $F = 2.06$, $df = 2/432$; time × diagnosis × APOE: $F = 2.34$, $df = 4/432$).

Table 1 Clinical characteristics of ILSE participants with AD, MCI, or cognitively healthy

	AD (A) $n = 22$	MCI (B) $n = 82$	Cognitively healthy (C) $n = 118$	χ^2, Tukey, ANOVA
Age (years)	74.8 ± 1.0	74.3 ± 1.1	74.0 ± 1.0	A > C***, B = C, A
% Female (*n*)	40.9 (9)	47.6 (39)	47.5 (56)	n.s.
% Statin use (*n*)	31.8 (7)	29.3 (24)	22.0 (26)	n.s.
% APOEε4 (*n*)	27.3 (6)	22.0 (18)	23.7 (28)	n.s.
Education (years), mean ± SD	11.2 ± 1.8	12.1 ± 2.2	13.7 ± 3.0	A, B < C***
MMSE, mean ± SD	24.2 ± 2.1	28.1 ± 1.4	28.9 ± 1.2	A < B < C***
Total cholesterol (mg/dl)				
VT1, mean ± SD	246.1 ± 40.7	247.0 ± 43.8	233.0 ± 38.0	B > C*
VT2, mean ± SD	242.8 ± 37.4	242.9 ± 37.5	232.6 ± 36.2	n.s.
VT3, mean ± SD	219.5 ± 47.1	221.3 ± 42.8	228.4 ± 38.9	n.s.
BMI				
VT1, mean ± SD	27.31 ± 2.99	26.71 ± 3.25	26.32 ± 3.82	n.s.
VT2, mean ± SD	28.58 ± 3.13	27.55 ± 3.84	27.30 ± 4.02	n.s.
VT3, mean ± SD	28.40 ± 4.42	27.22 ± 3.42	27.76 ± 4.61	n.s.

MCI mild cognitive impairment, *AD* Alzheimer's disease, *VT* visit time, *MMSE* mini-mental state examination, *BMI* body mass index

*** $p < 0.0001$; * $p < 0.05$

Table 2 Mean total cholesterol by APOEε4 status and diagnosis at VT3

	AD		MCI		Cognitive healthy	
	APOE4ε+ $n = 6$	APOEε4− $n = 16$	APOEε4+ $n = 18$	APOEε4− $n = 64$	APOEε4+ $n = 28$	APOEε4− $n = 90$
Total cholesterol (mg/dl), mean ± SD						
VT1	281.0 ± 42.0	232.9 ± 33.9	267.5 ± 57.9	241.1 ± 37.6	247.3 ± 46.8	228.6 ± 34.0
VT2	249.8 ± 40.3	240.1 ± 37.3	259.1 ± 42.6	238.4 ± 34.9	241.5 ± 35.1	229.8 ± 36.4
VT3	247.8 ± 54.2	208.9 ± 41.0	221.9 ± 44.5	221.2 ± 42.6	247.7 ± 40.7	222.4 ± 36.6
Repeated measures ANOVA						
Main effects						
Diagnosis ($df = 2$)						$F = 0.77$
APOE ($df = 1$)						$F = 14.39$**
Time ($df = 2$)						$F = 13.68$***
Interactions						
Diagnosis × APOE ($df = 2$)						$F = 0.50$
Time × diagnosis ($df = 4$)						$F = 5.40$**
Time × APOE ($df = 2$)						$F = 2.06$
Time × diagnosis × APOE ($df = 4$)						$F = 2.34$

The ILSE study

MCI mild cognitive impairment, *AD* Alzheimer's disease, *VT* visit time

** $p < 0.005$; *** $p < 0.0001$

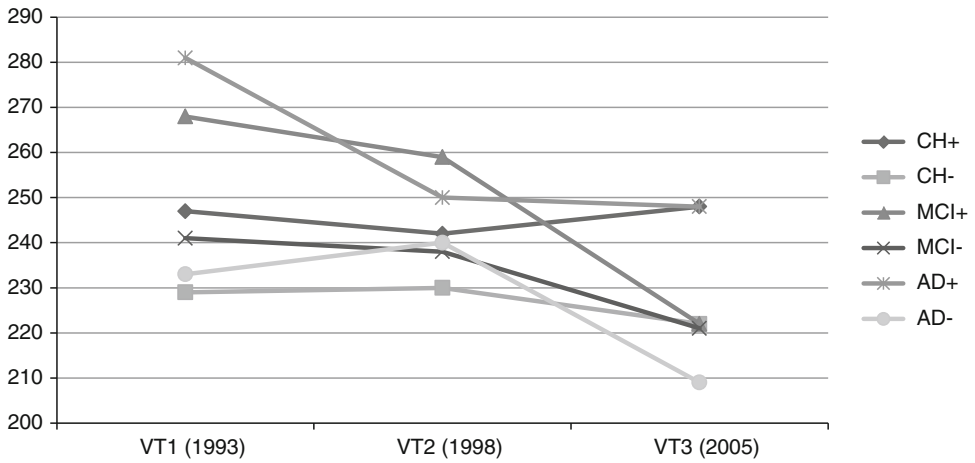

Fig. 2 Longitudinal course of total cholesterol means by diagnosis and APOE genotype. *VT* visit time, *CH* cognitive healthy, *MCI* mild cognitive impairment, *AD* Alzheimer's disease, + participants with APOEε4 allele, − participants without APOEε4 allele. VT1 (1993–1995) mean age = 62.4 (SD = 2.4), VT2 (1995–1998) mean age = 66.7 (SD = 1.1), VT3 (2005–2008) mean age = 74.3 (SD = 1.2)

A logistic regression revealed an increased risk of developing a cognitive disorder for the higher quartile of TC levels in comparison with the lower (OR 2.64, 95 % CI 1.12–6.23, $p < 0.05$) and second lower quartile (OR 3.15, CI 1.29–7.70, $p < 0.05$). TC levels at VT2 and VT3 were not associated with cognitive disorders.

Discussion

In our study, TC levels at age 60 were associated with a diagnosis of MCI or AD 14 years later. Moreover, in the course of 14 years, a distinct trajectory in TC levels among those who develop MCI or AD in comparison with subjects that remain healthy was observed, such that TC remained stable in the cognitively healthy, whereas decline was observed among those developing MCI or AD. Interestingly, these associations were independent of potential modulators of the cholesterol–dementia relationship, such as APOE genotype and statin use, as well as other potentially confounding variables, including cardiovascular risk factors (e.g., smoking, hypertension, BMI, and diabetes).

Our findings confirm a previous report by Solomon et al. [4] suggesting a bidirectional relationship between TC levels and MCI/AD diagnosis such that high TC levels in midlife is a risk factor for subsequent dementia, while an

observed decrease of TC levels after midlife may reflect ongoing disease processes. Thus, our results support findings [10] describing a more pronounced decline in TC among those who develop AD at least 15 years before a clinical diagnosis [26].

Study methodologies differ in this field, which may influence observations, as well as interpretation thereof. Some [2, 3, 27, 28]—but not all [26, 29]—studies show a relationship between higher TC values and subsequent development of MCI or dementia. Most studies relating TC to dementia are cross sectional or have included participants across a broad age range. This limits conclusions related to temporality of the association, since TC levels do not only change with age, but also with secular effects (e.g., birth cohort) [30]. It is well documented that TC levels increase with age, rising to a plateau before the age of 70 and subsequently decrease in older age (for an example in the German population see [31]). Since our study cohort is comprised of persons from the general German population born between 1930 and 1932, we have, by design, adjusted for age.

In addition, differences in diagnostic criteria exist. In a Finnish study [4], for example, only individuals scoring 24 or below on the MMSE were referred for subsequent diagnostic evaluation with respect to MCI or AD. This screening algorithm may have led to a selection of individuals who were in more advanced stages of MCI or dementia but excluded most cases of preclinical dementia. In our study, every participant underwent thorough neuropsychological testing, as did participants in the Gothenburg birth cohort studies [26, 32]. This made it possible to identify subjects in early stages of MCI who often score above 24 on the MMSE. In addition, we improved diagnostic accuracy via personal medical assessment of each participant focusing on medical and neuropsychiatric morbidities. Thus, given our inclusion- and exclusion criteria, our baseline sample was cognitively healthy. We were therefore able to chart the trajectory of TC levels in relation to MCI/AD overtime. In addition, participants with mild cognitive disorder due to a general medical condition (i.e., cardiovascular disease or severe metabolic disorder) or any psychiatric comorbidity (e.g., history of major depression or alcohol abuse) were excluded to assess the relationship of TC among participants with a specific MCI syndrome, and at risk for developing an AD type of dementia. This was accomplished in concordance with the Consensus of the International Psychogeriatric Association [17] which emphasizes that the differential diagnosis between MCI, dementia, and ICD-10 "mild cognitive disorder" should be considered the most important.

The prevalence of the APOEε4 allele was higher in those with AD versus those developing MCI or remaining cognitively healthy. That this difference did not reach statistical significance may be due to lack of power, to the low prevalence of AD in this age group and the relatively low APOEε4 prevalence. The Finnish population, however, has a higher APOEε4 allele prevalence, as well as higher average TC levels and lower MMSE scores [33]. These characteristics may well account for the strong TC-dementia observations in Finnish studies. Solomon et al. [4] found APOEε4 prevalence of 35 % in those without dementia or MCI, but over 50 % in those with dementia. Other European and American community-based studies yielded APOEε4 prevalences that are comparable with those observed in ILSE among cognitively healthy elderly: 11.2 % in a French study [34] and 12.6 % in white Americans [35]. These population differences emphasize potential difficulties in extrapolating results from one population to another even within Europe, and underscores the importance of conducting epidemiological studies in individual European countries in relationship to vascular factors in dementia etiology.

Selection effects have to be discussed as a potential confounding factor, since we solely focused on survivors from the ILSE. In Mielke et al. [32] the differences in TC levels reached significance only in the survivor analysis, and no significant differences appeared between the groups in the whole sample analysis. Nevertheless, our follow-up quote of 76 % of initial subjects in the 14 years with a strict and complete clinical examination of all study subjects in all visits expands the clinical significance of our findings.

To our knowledge, this is the first study describing the longitudinal relationship of APOE and TC in relation to clinically diagnosed MCI and AD. Considered together, APOE genotype and TC levels may be independent risk factors [2] for AD at the population level. One epidemiological study [36] found high TC levels to be a risk factor only among non-APOEε4 carriers, however, this study dichotomized their sample according to TC levels without reporting whether APOEε4 carriers had elevated TC levels in comparison with non-carriers [37–39]. Gender and statin use have to be considered as potential confounding factors. However, diagnostic groups showed only marginal, non-significant differences with respect to these variables; in addition, the proportion of subjects who received statins were rather low and did not exceed 33 %. Studies describing a protective effect of statins in cognitive disorders (AD and MCI) are misunderstanding, with some showing a protective effect [7, 40] and other showing no clear relation [41, 42]. Since most of them focused on dementia and very few in MCI as an outcome, more prospective studies are needed to address this topic.

In conclusion, higher TC levels at age 60 are associated with the development of MCI and AD at age 75 years (highest vs. lowest quartile: OR 2.64, 95 % CI 1.12–6.23, $p < 0.05$). While TC levels decline and stabilize during

this period, these effects are not accounted for by APOE genotype, birth cohort, statin treatment, or other cardiovascular risk factors. These findings support the hypothesis that preventive measures targeted on TC in relationship to cognition may have benefit before the seventh decade of life.

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Conflict of interest P. Toro, CH. Degen, D. Gustafson, M.Pierer, P. Schönknecht and J. Schröder declare no conflict of interest.

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Original Research Article

The *COMT p.Val158Met* Polymorphism and Cognitive Performance in Adult Development, Healthy Aging and Mild Cognitive Impairment

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Key Words
Catechol-O-methyltransferase · Cognitive capacity · Cognitive flexibility · Aging · Mild cognitive impairment · Dopamine

Abstract
Background: The impact of genetic polymorphisms on cognition is assumed to increase with age as losses of brain resources have to be compensated for. We investigate the relation of catechol-O-methyltransferase *(COMT) p.Val158Met* polymorphism and cognitive capacity in the course of adult development, healthy aging and the development of mild cognitive impairment (MCI) in two birth cohorts of subjects born between 1930 and 1932 or between 1950 and 1952. ***Methods:*** Thorough neuropsychological assessment was conducted in a total of 587 participants across three examination waves between 1993 and 2008. The *COMT* genotype was determined as a restriction fragment length polymorphism after PCR amplification and digestion with *Nla*III. ***Results:*** Significant effects of the *COMT p.Val158Met* polymorphism were identified for attention and cognitive flexibility in the younger but not the older cohort. ***Conclusion:*** These results confirm the importance of the *COMT p.Val158Met* genotype on tasks assessing attention and cognitive flexibility in midlife but not in healthy aging and the development of MCI. Our findings suggest that the influence of *COMT* changes as a function of age, decreasing from midlife to aging.

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Introduction

Cognitive functioning is commonly linked to dopaminergic activity, and studies have associated age-related losses of dopaminergic activity with age-related cognitive decline [1]. Catechol-O-methyltransferase *(COMT)* is involved in the modulation of dopamine in the prefrontal cortex (PFC), where its resultant enzyme operates postsynaptically by inactivating neurotransmission [2, 3]. *COMT* contains a functional polymorphism, which enables the substitution of valine *(Val)* with methionine *(Met)*. The *Met* allele results in the production of an enzyme that is unstable at body temperature with approximately ¼ of the activity of the *Val* polypeptide [4]. Thus, the *Met* allele is associated with lower enzyme activity, elevated dopamine levels and superior cognitive performance, while the *Val* allele is associated with higher enzyme activity, reduced dopamine levels and inferior cognitive performance [5, 6]. While feasible, the association of *COMT* and cognitive capacity has been the focus of debate. Some studies identified an association, such that individuals with a homozygous *Met/Met* genotype exhibit increased efficiency and superior performance on tests of executive functions and working memory in comparison to individuals with a homozygous *Val/Val* genotype [4, 7, 8]. By contrast, other studies, including a meta-analysis [9], describe this association to be rather limited or even absent [for a review, see 5; 8, 10].

The respective discrepancies may partially arise from differential effects of the *COMT* polymorphism on cognitive capacity during midlife development and old age. It has been put forward that the impact of genetic polymorphisms on cognitive capacity increases when resources decline – as is the case in aging [11]. As such, a decline in anatomical and neurochemical brain resources may lead to subsequent decline in compensatory skills, thereby amplifying genetic effects on cognitive capacity. In line with this are results reported by Nagel et al. [12] who found a negative effect of the *Val* allele on the number of perseverative errors in the Wisconsin Card Sorting Test in older (between 60 and 70 years of age), but not younger (between 20 and 30 years of age), participants. However, longitudinal studies yield contradictory results. De Frias et al. [13] found *Val/Val* carriers' performance on tasks of executive functioning to decline over a 5-year interval in contrast to *Met* carriers and identified a *COMT* × age interaction for middle-aged adults (50–60 years). Fiocco et al. [14] found the opposite, such that individuals (aged 70–79) with a homozygous *Val/Val* genotype displayed significantly less decline in the performance on the Digit Symbol Substitution Test than carriers of the *Met/Met* genotype across an 8-year interval, indicating an approximation of genotypes in cognitive test performance in aging. Other longitudinal studies have found no genetic impact on cognitive decline (n = 53, mean age 75.5, SD = 5.3 [15]; n = 473, age 64–68 [16]).

No overall conclusion can be drawn based on the respective studies, as they differ regarding their overall design, neuropsychological instruments, age of subjects, and length of follow-up interval. Previous research has focused on cognitive domains most commonly linked to PFC activity, e.g. executive functioning and working memory [4, 17], while from a neuropsychological standpoint, the inclusion of other cognitive domains relying on PFC activation (e.g. episodic memory retrieval [18]) is important to investigate the domain specificity of the respective effects. Here, we sought to examine the role of the *COMT p.Val158Met* genotype in different aspects of cognitive capacity in the course of adult development, healthy aging and the development of mild cognitive impairment (MCI) in the Interdisciplinary Longitudinal Study on Adult Development and Aging (ILSE), which involves two large birth cohorts of subjects born between 1930 and 1932 (C30) or between 1950 and 1952 (C50) [19, 20]. We hypothesized that there are specific effects of *COMT p.Val158Met* polymorphism on tests of executive functioning, which are more pronounced in older than in younger subjects.

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Methods

Participants

Participants were recruited via local registries. For the purpose of the present study, exclusion criteria were psychiatric diagnoses affecting cognitive functioning apart from MCI, as defined by the Aging-Associated Cognitive Decline criteria [21]. Participants with mild cognitive disorder due to a medical condition, manifest Alzheimer's disease, other forms of dementia, or mood disorders were excluded. Examinations of both birth cohorts were conducted in parallel.

Measures

The first examination (T1) took place in 1993/1994, the second examination (T2) in 1998/1999, and the third examination (T3) was conducted between 2006 and 2008. Each time, careful screening of physical and mental health using extensive physical examination and the German version of the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders [22] was performed by trained physicians. DNA was extracted at T3 from whole blood using the Nucelon® Genomic DNA Extraction Kit BACC1. The *COMT* genotype was determined as a restriction fragment length polymorphism after PCR amplification and digestion with *Nla*III, as described by Lachman et al. [23]. The same kit was used for both cohorts.

To assess cognitive capacity, the subtests Word List (WL) and Digit Symbol Test (DST) of the Nuremberg Age Inventory [24], the subtests Mosaic Test (MT) and Finding Similarities (FS) of the Wechsler Intelligence Test Battery [25], the subtests Word Fluency (WF) and Visual Thinking (VT) of the Performance Evaluation System [26] as well as the Attentiveness Endurance Test 'd2' (D2)[27] were administered. Due to time restrictions, certain subtests were not administered to the younger birth cohort at T2.

Statistical Analyses

Statistical analyses were performed using the SPSS 14.0 statistical package. After data description, analyses of variance (ANOVAs) and χ^2 tests were conducted to test for significant differences between (a) cohorts, healthy participants and participants with MCI, and (b) carriers of different *COMT p.Val158Met* genotypes. Afterwards, repeated-measures ANOVAs were performed with test scores at all three examination waves being treated as repeated measures. A separate analysis was conducted for healthy individuals using cohort (C30/C50) and *COMT* genotypes as independent variables and controlling for the level of education. Afterwards, C30 was analyzed separately to allow for the inclusion of cognitive status (MCI) in the model. In case assumption of sphericity was violated, Greenhouse-Geisser corrected values were used. The Bonferroni correction was applied to correct for multiple testing.

Results

Demographics and Baseline Characteristics

A total of 587 participants were included in the analysis: 188 healthy individuals from C30, 93 individuals diagnosed with MCI from C30, and 306 healthy individuals from C50, respectively. Distribution of genotypes was consistent with the Hardy-Weinberg equilibrium (*Val/Val* = 21.98%, *Val/Met* = 52.30%; *Met/Met* = 25.72%; $\chi^2 = 1.32$; $p = 0.251$). Demographic and baseline characteristics across genotypes can be inferred from table 1, while demographic characteristics across cohorts can be inferred from table 2.

Cognition

Results of the repeated-measures ANOVAs are presented in table 3. For the healthy participants from C30 and C50, no significant main effect of the *COMT* genotype on cognitive performance was identified. Cohort effects were evident for subtests DST (mean difference = –6.897; SE = 0.847; $p < 0.001$), MT (mean difference = –2.467; SE = 0.716; $p = 0.001$), VT (mean difference = –2.103; SE = 0.532; $p < 0.001$), WL (mean difference = –1.783; SE = 0.236; $p < 0.001$), D2 (mean difference = –14.888; SE = 7.023; $p = 0.035$) and FS (mean difference = –0.814; SE = 0.323; $p = 0.012$), with C50 performing better than C30. A significant inter-

Table 1. Demographic characteristics and baseline cognitive performance across the *COMT* genotype

	Val/Val (n = 129)	Val/Met (n = 307)	Met/Met (n = 151)	ANOVA/χ^2	Duncan
Demographics					
Age baseline	52.98 (9.30)	52.78 (9.43)	53.72 (9.28)	F = 0.521, p = 0.594	
Education	13.71 (2.74)	13.94 (2.77)	13.95 (2.74)	F = 0.382, p = 0.683	
APOE genotype (% ε4 allele)	19.38	25.41	18.54	χ^2 = 3.39, d.f. = 2 p = 0.183	
Sex (% females)	48.83	48.86	47.68	χ^2 = 0.062, d.f. = 2 p = 0.970	
Cohort (% C30)	47.29	46.58	50.99	χ^2 = 0.813, d.f. = 2 p = 0.666	
Cognitive status (% MCI)	15.50	15.64	16.55	χ^2 = 0.079, d.f. = 2 p = 0.961	
Cognitive performance baseline					
DST	48.29 (10.95)	51.10 (10.31)	50.61 (10.02)	F = 3.40, p = 0.034	Val/Val < Val/Met, Met/Met
MT	29.77 (8.07)	30.68 (8.44)	30.73 (8.13)	F = 0.632, p = 0.532	
WL	12.70 (3.16)	12.76 (3.40)	12.81 (3.35)	F = 0.038, p = 0.963	
D2	147.51 (42.52)	156.21 (33.12)	159.19 (38.85)	F = 3.77, p = 0.023	Val/Val < Val/Met, Met/Met
FS	26.02 (4.33)	26.70 (4.17)	26.43 (4.18)	F = 1.204, p = 0.301	
WF	31.26 (7.98)	33.11 (9.00)	32.40 (9.28)	F = 2.008, p = 0.135	
VT	23.33 (6.69)	24.27 (6.19)	24.38 (5.96)	F = 1.242, p = 0.290	

Figures in parentheses are SD.

Table 2. Demographic characteristics and baseline cognitive performance across cohorts and cognitive status groups

	Healthy		MCI C30 (n = 93)	ANOVA/χ^2	Duncan
	C30 (n = 188)	C50 (n = 306)			
Demographics					
Mean age baseline ± SD, years	62.78±0.897	44.15±0.904	62.76±0.877	F = 31525.63, p < 0.001	Healthy C50 < Healthy C30, MCI
Mean education ± SD, years	13.77±3.027	14.47±2.525	12.25±2.170	F = 25.48, p < 0.001	Healthy C50 > Healthy C30 > MCI
APOE genotype (% ε4 allele)	22.34	22.55	21.51	χ^2 = 0.03, d.f. = 2, p = 0.983	
Sex (% females)	53.19	46.08	47.31	χ^2 = 2.427, d.f. = 2, p = 0.297	

action of *COMT* and cohort arose for DST, suggesting that an effect of the *COMT* genotype on executive functioning was apparent in C50 but not in C30 (fig. 1). A triple interaction of the *COMT* genotype, time and cohort was found for MT as illustrated in figure 2.

The second repeated-measures analysis examined the influence of the *COMT* genotype and diagnosis of MCI on cognitive performance over 14 years in C30. No significant effects of the *COMT* genotype appeared. Significant main effects of diagnosis (MCI/cognitively healthy) emerged for DST (mean difference = 8.018; SE = 2.252; p < 0.001), MT (mean difference = 3.693; SE = 0.934; p < 0.001), VT (mean difference = 2.689; SE = 0.770; p = 0.001), WF (mean difference = 4.902; SE = 1.091; p < 0.001), WL (mean difference = 1.427; SE = 0.325; p < 0.001),

Table 3. Results of the repeated-measures analyses

Test	Healthy subjects C30 and C50	C30 (healthy and MCI)
DST	COMT: $F_{2, 451} = 1.149$, p = 0.318 Cohort: $F_{1, 451} = 66.263$, p < 0.001 COMT × cohort: $F_{2, 451} = 3.326$, p = 0.037 Time: $F_{2, 902} = 0.258$, p = 0.772 COMT × time: $F_{4, 902} = 0.152$, p = 0.962 Time × cohort: $F_{2, 902} = 9.765$, p < 0.001 Time × COMT × cohort: $F_{4, 902} = 0.522$, p = 0.719	COMT: $F_{2, 249} = 0.972$, p = 0.380 Diagnosis: $F_{1, 249} = 4.992$, p < 0.001 COMT × diagnosis: $F_{2, 249} = 1.8$, p = 0.167 Time: $F_{2, 498} = 0.653$, p = 0.521 COMT × time: $F_{4, 498} = 0.701$, p = 0.591 Time × diagnosis: $F_{2, 498} = 0.951$, p = 0.387 Time × COMT × diagnosis: $F_{4, 498} = 1.453$, p = 0.215
MT	COMT: $F_{2, 454} = 0.288$, p = 0.750 Cohort: $F_{1, 454} = 11.885$, p = 0.001 COMT × cohort: $F_{2, 454} = 0.871$, p = 0.419 Time: $F_{1, 454} = 0.053$, p = 0.817 COMT × time: $F_{2, 454} = 1.162$, p = 0.314 Time × cohort: $F_{1, 454} = 8.350$, p = 0.004 Time × COMT × cohort: $F_{2, 454} = 4.909$, p = 0.008	COMT: $F_{2, 250} = 0.622$, p = 0.538 Diagnosis: $F_{1, 250} = 14.156$, p < 0.001 COMT × diagnosis: $F_{2, 250} = 1.113$, p = 0.330 Time: $F_{2, 500} = 3.561$, p = 0.029 COMT × time: $F_{4, 500} = 2.127$, p = 0.076 Time × diagnosis: $F_{2, 500} = 0.267$, p = 0.766 Time × COMT × diagnosis: $F_{4, 500} = 0.572$, p = 0.683
VT	COMT: $F_{2, 453} = 1.820$, p = 0.163 Cohort: $F_{1, 453} = 15.644$, p < 0.001 COMT × cohort: $F_{2, 453} = 0.143$, p = 0.867 Time: $F_{1, 453} = 2.170$, p = 0.141 COMT × time: $F_{2, 453} = 0.265$, p = 0.767 Time × cohort: $F_{1, 453} = 20.291$, p < 0.001 Time × COMT × cohort: $F_{2, 453} = 0.536$, p = 0.585	COMT: $F_{2, 249} = 1.572$, p = 0.210 Diagnosis: $F_{1, 249} = 15.193$, p < 0.001 COMT × diagnosis: $F_{2, 249} = 0.277$, p = 0.758 Time: $F_{2, 498} = 3.692$, p = 0.026 COMT × time: $F_{4, 498} = 1.976$, p = 0.097 Time × diagnosis: $F_{2, 498} = 2.942$, p = 0.054 Time × COMT × diagnosis: $F_{4, 498} = 0.681$, p = 0.605
WF	COMT: $F_{2, 453} = 1.247$, p = 0.288 Cohort: $F_{1, 453} = 0.003$, p = 0.960 COMT × cohort: $F_{2, 453} = 0.367$, p = 0.693 Time: $F_{1, 453} = 3.352$, p = 0.068 COMT × time: $F_{2, 453} = 1.199$, p = 0.302 Time × cohort: $F_{1, 453} = 9.596$, p = 0.002 Time × COMT × cohort: $F_{2, 453} = 2.766$, p = 0.064	COMT: $F_{2, 248} = 0.334$, p = 0.717 Diagnosis: $F_{1, 248} = 24.633$, p < 0.001 COMT × diagnosis: $F_{2, 248} = 0.264$,p = 0.768 Time: $F_{2, 496} = 0.410$, p = 0.664 COMT × time: $F_{4, 496} = 0.971$, p = 0.423 Time × diagnosis: $F_{2, 496} = 7.027$, p = 0.001 Time × COMT × diagnosis: $F_{4, 496} = 1.188$, p = 0.315
WL	COMT: $F_{2, 451} = 0.622$, p = 0.537 Cohort: $F_{1, 451} = 57.018$, p < 0.001 COMT × cohort: $F_{2, 451} = 1.614$, p = 0.200 Time: $F_{2, 902} = 0.225$, p = 0.798 COMT × time: $F_{4, 902} = 0.602$, p = 0.661 Time × cohort: $F_{2, 902} = 0.546$, p = 0.580 Time × COMT × cohort: $F_{4, 902} = 0.616$, p = 0.651	COMT: $F_{2, 250} = 0.689$, p = 0.503 Diagnosis: $F_{1, 250} = 26.705$, p < 0.001 COMT × diagnosis: $F_{2, 250} = 0.095$, p = 0.909 Time: $F_{2, 500} = 9.960$, p < 0.001 COMT × time: $F_{4, 500} = 0.099$, p = 0.983 Time × diagnosis: $F_{2, 500} = 3.969$, p = 0.019 Time × COMT × diagnosis: $F_{4, 500} = 1.865$, p = 0.115
D2	COMT: $F_{2, 442} = 1.237$, p = 0.291 Cohort: $F_{1, 442} = 4.494$, p = 0.035 COMT × cohort: $F_{2, 442} = 1.269$, p = 0.282 Time: $F_{2, 884} = 12.590$, p < 0.001 COMT × time: $F_{4, 884} = 0.152$, p = 0.962 Time × cohort: $F_{2, 884} = 31.965$, p < 0.001 Time × COMT × cohort: $F_{4, 884} = 0.823$, p = 0.511	COMT: $F_{2, 241} = 0.355$, p = 0.702 Diagnosis: $F_{1, 241} = 32.309$, p < 0.001 COMT × diagnosis: $F_{2, 241} = 0.802$, p = 0.450 Time: $F_{2, 482} = 9.122$, p < 0.001 COMT × time: $F_{4, 482} = 0.992$, p = 0.412 Time × diagnosis: $F_{2, 482} = 1.143$, p = 0.320 Time × COMT × diagnosis: $F_{4, 482} = 0.556$, p = 0.694
FS	COMT: $F_{2, 454} = 0.013$, p = 0.987 Cohort: $F_{1, 454} = 6.353$, p = 0.012 COMT × cohort: $F_{2, 454} = 0.09$, p = 0.914 Time: $F_{1, 454} = 0.140$, p = 0.709 COMT × time: $F_{2, 454} = 1.561$, p = 0.211 Time × cohort: $F_{1, 454} = 1.899$, p = 0.169 Time × COMT × cohort: $F_{2, 454} = 0.339$, p = 0.713	COMT: $F_{2, 251} = 1.147$, p = 0.319 Diagnosis: $F_{1, 251} = 31.246$, p < 0.001 COMT × diagnosis: $F_{2, 251} = 0.298$, p = 0.743 Time: $F_{2, 502} = 4.832$, p = 0.008 COMT × time: $F_{4, 502} = 2.352$, p = 0.053 Time × diagnosis: $F_{2, 502} = 3.814$, p = 0.023 Time × COMT × diagnosis: $F_{4, 502} = 1.784$, p = 0.131

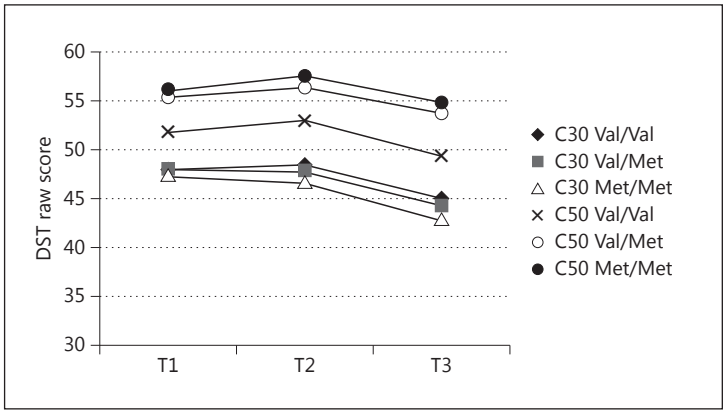

Fig. 1. Interaction of *COMT* and cohort for DST.

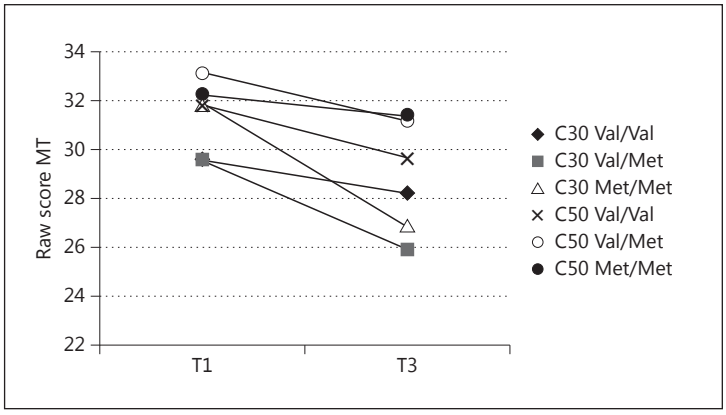

Fig. 2. Interaction of *COMT*, cohort and time for MT.

D2 (mean difference = 57.102; SE = 10.046; $p < 0.001$), and FS (mean difference = 2.920; SE = 0.515; $p < 0.001$), with healthy individuals outperforming those diagnosed with MCI. No interaction of the *COMT* genotype and diagnosis was found. No significant interaction effects of the *COMT* genotype and time appeared, while interaction effects by trend emerged for MT, VT, and FS. Significant interaction effects of time and diagnosis of MCI were found for WF, WL, and FS. No triple interaction was observed.

Discussion

In this study, we investigated the effect of the *COMT p.Val158Met* polymorphism on cognitive performance in a sample of 587 participants of two distinct age cohorts, born between 1930 and 1932 (C30) or between 1950 and 1952 (C50). Our results suggest that the *COMT* genotype exerts a different influence on cognitive functioning for the C50 than for the C30 cohort. For C50, we find significant differences in baseline test performance between *COMT* genotypes on the subtests D2 and DST, such that homozygous *Val* carriers perform more slowly than heterozygotes and homozygous *Met* carriers. We identified an interaction suggesting that this effect is only applicable to C50, but not to C30, contrary to our second hypothesis. The minimal effects of the *COMT p.Val158Met* polymorphism are more pronounced in tests of executive functioning than other cognitive domains. However, no interaction with time was identified, indicating that the *COMT* genotype does not influence cognitive trajectories over time. An individual analysis for the C30 cohort suggests that cognitive perfor-

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mance trajectories in older subjects are largely independent on the *COMT* genotype. No effect was found for subjects diagnosed with MCI. These results confirm the importance of the *COMT p.Val158Met* genotype on tasks assessing attention and cognitive flexibility in midlife but not in healthy aging and the development of MCI.

De Frias et al. [13] found that the performance of *Val/Val* carriers on tasks of executive functioning declined over a 5-year interval compared to that of *Met* carriers. An interaction of *COMT* and age was identified for middle-aged participants (aged 50–60), supporting the idea that discrepancies due to genetic effects are greater in midlife than in aging. Fiocco et al. [14] identified a difference in cognitive decline across an 8-year interval, such that individuals with a homozygous *Val/Val* genotype displayed significantly less decline in the Digit Symbol Substitution Test performance compared to *Met* homozygotes, indicating an approximation of different genotypes in test performance in the course of healthy aging. Generally, the respective studies are in line with our findings, even though we did not identify an interaction effect of time (age) and the *COMT* genotype. However, a few studies point to a potential amplification of genetic effects in old age [12]. While it is plausible that losses of brain resources such as decline of striatal and extrastriatal dopamine or atrophy affecting the PFC may amplify the effects of genetic polymorphisms such as *COMT p.Val158Met* on cognition [11], our results show that the *COMT* genotype on its own is not a determining factor. Further studies have demonstrated inefficient cortical processing as reflected by low performance and greater activity in *Val* homozygotes compared to *Met* homozygotes in tasks demanding working memory capacity in participants in their mid-thirties [4, 28] and attentional control [29]. Remarkably, neurological differences were sometimes identified in the absence of effects on behavioral measures such as test performance [28], suggesting a compensatory mechanism. Since we did not find an effect of the *COMT* genotype on cognitive trajectories, we must consider that certain factors related to the birth cohort are determinative rather than age per se.

A potential limitation to studies examining specific cognitive domains is their reliance on neuropsychological test batteries that are largely classified by their content. Assessment instruments can only partially reflect differential cognitive domains or phenotypes (for a review, see Harris and Deary [30]). Moreover, an interplay of different candidate genes affecting dopamine regulation seems likely. There exists relatively robust evidence for risk of increased cognitive decline from *APOE ε4* allele as well as *BDNF* [30, 12]. However, in this study, we were able to consider a follow-up interval of 14 years, allowing for conclusions on the influence of the *COMT* genotype on the process of healthy aging and the development of MCI, while previous research was limited to a few years only. Moreover, directly contrasting two different birth cohorts allowed us to delineate cohort effects from aging effects. Our findings can shed light on the often somewhat contradictory findings reported in the literature. Another strength of this study is the use of extensive neuropsychological testing. Given the role of the *COMT* genotype in dopaminergic pathways, it is likely that areas relying on the PFC are affected differently than other areas. Results of our study suggest that the *COMT p.Val158Met* polymorphism has a larger genetic contribution to tests of attention, cognitive flexibility and information processing speed at ages 43–56 than at ages 63–76. The effects of *COMT* were therefore specific to tests assessing executive functioning rather than tests of memory, verbal fluency or visuospatial thinking.

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Diabetes mellitus Type II and cognitive capacity in healthy aging, mild cognitive impairment and Alzheimer's disease

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ABSTRACT

While diabetes mellitus (DM) Type II has repeatedly been linked to Alzheimer´s disease (AD) and mild cognitive impairment (MCI), longitudinal research is scarce and disease duration has not always been taken into account. In a birth cohort born between 1930 and 1932 we investigated the influence of DM Type II and disease duration on neuropsychological functioning (memory/learning, attention, verbal fluency, visuospatial thinking and abstract thinking) across 14 years. Subjects who developed MCI or AD performed significantly poorer on all neuropsychological tests applied. While significant main effects DM Type II did not arise, its presence led to a significant deterioration of performance in the digit symbol test and visuospatial thinking over time. Additionally, in visuospatial thinking this change was more pronounced for individuals suffering from MCI/AD. We found that, as a concomitant disease DM Type II does not affect memory functioning, which is typically compromised in MCI and early AD. Rather, it may lead to deficits in cognitive flexibility and visuospatial thinking. DM Type II can be considered a frequent comorbid condition which can aggravate the course of MCI and AD. In this respect it may serve as a model for other comorbid conditions in AD.

1. Introduction

Projections of future Diabetes Mellitus (DM) prevalence estimate a global increase of diagnoses especially in people aged 65 and older (Wild et al., 2004). Cross-sectional and longitudinal studies have identified associations of DM Type II and cognitive impairment (Strachan et al., 1997; Awad et al., 2004), incidence of mild cognitive impairment (MCI), and dementia (Stewart and Liolitsa, 1999). Various mechanisms have been proposed by which DM Type II affects cognitive functioning and aggravates the clinical picture typically observed in MCI and dementia. However, the complex interrelationship between diabetes and dementia are most likely confounded and/or mediated by vascular, nonvascular and genetic risk factors. A previous study conducted by our research group showed that DM Type II is associated with psychomotor slowing, but does not affect other cognitive domains, including memory (Toro et al., 2009). However, reviews by Awad et al. (2004) and Strachan et al. (1997) suggest that deficits are most consistently found in verbal memory and processing speed, while other cognitive domains (visuospatial functioning, attention, language) remain largely preserved. A detailed review of longitudinal studies by Cukierman et al. (2005) suggests a 1.2–1.5 fold greater decline in cognitive functioning and a 1.6 greater risk for developing dementia in individuals diagnosed with DM Type II compared to healthy controls. Studies typically comprised follow-up intervals between 2 and 6 years limiting generalizability to healthy aging and the development of dementia. Thus, it remains unclear how cognition is affected and how different cognitive domains progress over time in the presence of DM Type II.

It was the aim of this study to examine a) the influence of DM Type II on cognitive functioning in healthy aging, b) the influence of DM Type II on cognitive functioning in the development of MCI/AD, and c) potential associations of cognitive trajectories with disease duration. Our analyses are based on data from the Interdisciplinary Longitudinal Study of Adult Development and Aging (ILSE, Pantel et al., 2003; Schönknecht et al., 2005) – a prospective,

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population-based study with a 14 year follow-up interval. Neuropsychological functioning was operationalized using the International Psychogeriatric Association working Party's (Levy, 1994) guidelines to establish the diagnosis of Aging Associated Cognitive Decline: memory/learning, attention, verbal fluency, visuo-spatial functioning and abstract thinking. In line with existing reviews and our previous study we expected that a) DM Type II is associated with decrements to cognitive functioning in healthy aging specifically pertaining to tasks assessing psychomotor speed, b) DM Type II aggravates the clinical picture typically observed in the development of MCI/AD and c) disease duration is negatively associated with cognitive functioning, such that individuals suffering from DM Type II for a longer period of time display larger decrements to cognitive functioning than individuals who have been diagnosed with DM Type II more recently.

2. Methods

2.1. Procedure

The ILSE is a longitudinal study which has followed up two birth cohorts, born between 1930 and 1932 or between 1950 and1952, for a total of 14 years. Participants were recruited via community registers in the Heidelberg and the Leipzig area, yielding a representative sample of N=1002 participants. The first examination (T1) took place in 1993/1994, the second examination (T2) in 1998/1999 and the third examination (T3) was conducted between 2006 and 2008. The fourth examination is about to be completed in 2016. Each time, careful screening of physical and mental health using extensive physical examination and the German version of the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R, Wittchen et al., 1991) was performed by trained physicians. Laboratory tests included triglycerides and glycosylated hemoglobin. Moreover, the Apolipoprotein E (APOE) genotype was determined using the LightCycler technology (Aslanidis and Schmitz, 1999).

Verbal memory was assessed using the subtest Word List of the Nuremberg Age Inventory (Oswald and Fleischmann, 1991), where twelve words are read to the participant, who is instructed to recall as many words as possible immediately after presentation and after a short delay period. Processing speed and mental flexibility were assessed using the digit symbol test of the Nuremberg Age Inventory (Oswald and Fleischmann, 1991), where participants are instructed to fill in symbols following a prescribed pattern as fast as possible. Visuospatial reasoning and abstract thinking capacity were assessed using the subtest Mosaic Test of the Wechsler Intelligence Test Battery (Tewes, 1991), where participants are asked to construct patterns using a set of duo-colored building blocks. The subtest Finding Similarities of the Wechsler Intelligence Test Battery (Tewes, 1991) assesses abstract reasoning capacity by asking participants to find similarities between a maximum of 16 word pairs. Verbal fluency is measured using the subtest verbal fluency of the "Leistungsprüfsystem" (Sturm et al., 1993) where participants are asked to name as many words as possible with the initial letter "s" or "f". Likewise, in the subtest visual thinking (Sturm et al., 1993) participants are presented two-dimensional pictures of three-dimensional geometrical figures and are asked to count the number of surfaces. Finally, the attentiveness endurance test "d2" (Brickenkamp, 1978) assesses attention and processing speed by instructing participants to identify whenever the letter "d" is presented in combination with 2 lines in several rows of distractor stimuli.

Basic visual screening was conducted prior to neuropsychological testing.

2.2. Participants

381 participants from the cohort born between 1930 and 1932 returned for T3 (Fig. 1). Participants meeting ICD-10 criteria for Mild Cognitive Disorder including the presence of systemic physical disorders known to cause cerebral dysfunction (World Health Organization, 1993), NINDS-AIREN criteria for Vascular Dementia (Roman et al., 1993), depression or anxiety disorders (DSM-III-R criteria; Wittchen et al., 1991) at any examination wave were excluded, leaving a final sample of 295 participants. 135 were diagnosed with MCI (International Psychogeriatric Association working Party; Schröder et al., 1998) or AD (McKhann et al., 1984), while 160 were healthy control subjects. A distinction between individuals with MCI and those with Mild Cognitive Disorder was drawn to delineate subjects at risk of developing AD from individuals with impairments resulting from vascular dysfunction and other systemic causes. DM Type II was diagnosed on the basis of laboratory tests (glycated hemoglobin levels) and subjects' histories. When necessary, medical records were called upon. Disease duration was assessed using self-report and medical records presented at T1. Individuals were then classified according to whether DM Type II had been present at T1 or not.

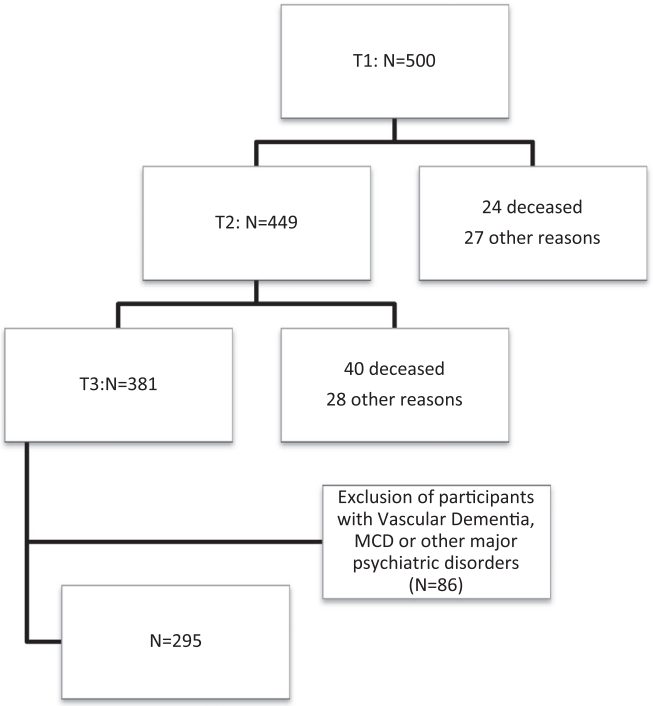

Fig. 1. Participant flow.

2.3. Statistical Analysis

Statistical Analysis System software (SAS Institute, Cary, NC; USA) was used for all statistical analyses. The level of significance was set to p=.05. Chi-Square test was used to compare individuals who dropped out between T1 and T3 and individuals who were included in the follow-up analysis. Afterwards, ANOVAs and Chi-Square tests were used to compare demographic and clinical characteristics between diagnostic groups at T3. Separate repeated measures ANOVAS (Proc GLM) were performed for each neuropsychological test using time between examination waves as the within subject factor, DM Type II (not present, present at T3 and T1, only present at T3) and diagnosis of MCI/AD as between subject factors. If assumption of sphericity was violated, Greenhouse-Geisser corrected values were used.

3. Results

Clinical and demographic characteristics of participants can be seen in Table 1. Note that at T3, individuals with MCI/AD without DM Type II were slightly older than the other participants ($F=3.97$, $p=.009$). Moreover, cognitively healthy participants had significantly more years of education than those with MCI/AD ($F=8.17$, $p<.0001$). As expected, individuals diagnosed with DM Type II showed increased glycosylated hemoglobin levels compared to those without DM Type II ($F=31.66$, $p<.0001$). Chi Square test revealed no difference in distribution of DM Type II in the MCI/AD and healthy control groups ($\chi^2=0.699$, $p=.403$). Note however, that the prevalence of DM Type II in participants diagnosed with MCI/AD is slightly higher with 20.61% as opposed to 16.25% in those without a diagnosis of MCI/AD. No association between APOE genotype and DM Type II was identified ($\chi^2=1.959$, $p=.162$). When comparing individuals that dropped out between T1 and T3 (N=119) and those that were included in this analysis (N=295) we found that 50% of individuals with a record of DM Type II at T1 (N=44) did not participate in T3, while the dropout rate for those that were not diagnosed with DM Type II was 26.10% ($\chi^2=10.964$, $p=.0009$).

Repeated measures analyses were performed controlling for age and education. An overview of results can be seen in Table 2.

Table 1
Comparison of demographic and clinical characteristics.

	MCI/AD with DM Type II N=27 (a)	MCI/AD without DM Type II N=104 (b)	Controls with DM Type II N=26 (c)	Controls without DM Type II N=134 (d)	F/χ^2	p	Duncan
Age T1	62.37 (1.04)	62.62 (.89)	62.42 (.86)	62.40 (.97)	F=1.31	0.271	
Age T2	66.42 (1.07)	66.76 (94)	66.56 (.96)	66.46 (1.02)	F=2.32	0.075	
Age T3	74.22 (1.01)	74.55 (1.17)	74.23 (0.99)	74.10 (1.12)	F=3.97	0.009	b ≥ a,c,d
Education	12.26 (2.23)	12.03 (2.18)	14.00 (2.71)	13.63 (3.08)	F=8.17	<.0001	a,b < c,d
T3HbA1c	6.68 (.78)	5.75 (52)	6.97 (1.48)	5.73 (.44)	F=31.66	<.0001	a,c > b,d
APOE (ε4 present %)	19.23	23.66	25.83	12.00	χ^2=2.47	0.480	

The analyses yielded no significant main effects of DM Type II, but an interaction effect of time (between examination waves) and DM Type II for the digit symbol test ($F_{4,468}$=3.23, p=.012) suggesting a steeper decline in cognitive performance for individuals diagnosed with DM Type II at T1 and T3, relative to individuals diagnosed with DM Type II at T3 only and those that do not have DM Type II (Fig. 2). The same pattern was observed for visual thinking ($F_{4,466}$=2.76, p=.027). Moreover, a triple interaction of time (between examination waves), DM Type II and diagnosis of MCI/AD was observed for visual thinking ($F_{4,464}$=2.81, p=.025) as illustrated in Fig. 3. Accordingly, individuals with both MCI/AD and DM Type II exhibited a steeper decline between T1 and T3 than other individuals. Moreover, performance of individuals with MCI/AD and DM Type II diagnosis at T3 (but not T1) exhibited a more pronounced decline between T1 and T2 than those with MCI/AD and not DM Type II.

4. Discussion

In the present study we investigated the putative influence of DM Type II on cognitive functioning in healthy aging and the development of MCI/AD. We found that individuals diagnosed with MCI/AD showed a slightly higher prevalence of DM Type II, even though the association did not reach statistical significance. As expected, cognitively healthy individuals performed better on tests of neuropsychological functioning than subjects diagnosed with MCI/AD across domains. Repeated measures analyses revealed no significant main effect of DM Type II on cognitive performance. For visual thinking and digit symbol subtests significant interaction effects of time between examination waves and DM Type II were identified suggesting a steeper decline in cognitive performance for individuals diagnosed with DM Type II at T1 already in comparison to those diagnosed with DM Type II only at T3 and those without DM Type II. Moreover, a triple interaction of time between examination waves, DM Type II and diagnosis for the subtest visual thinking suggests that performance decline of individuals suffering from MCI/AD and DM Type II (present at T1 already) is significantly more pronounced than in the other participants. Thus, we find that DM Type II aggravates cognitive decline observed in healthy aging in particular pertaining to tasks assessing processing speed, mental flexibility and visual thinking if DM Type II has been present for a longer period of time. Moreover, additional cognitive decrements in patients with MCI/AD are observed given relatively longer disease duration.

Our findings are in line with a previous cross-sectional study from our research group, where an influence of DM Type II was found for tasks assessing psychomotor speed and executive functioning (Trail-Making Test A & B), but not for memory and learning (Wechsler Memory Scale, logical memory) or global cognitive performance (Mini Mental State Examination) (Toro et al., 2009). Likewise, van den Berg et al. (2010) found modest alterations of cognitive functioning in participants diagnosed with DM Type II that were largely in the lines of "normal aging". The authors investigated domain-specific cognitive capacity (abstract reasoning, memory, information processing speed, attention and executive functioning and visuoconstruction) in 106 participants over a period of 4.1 years. They identified a significant effect of DM Type II on information processing speed and attention/executive functioning. However, no time*DM Type II interaction was observed. The authors concluded that diabetes-related cognitive changes progress slowly and over a prolonged period of time. Here, we were able to draw on a larger sample and a longer interval of inspection than van den Berg et al. (2010), and found support for the assumption that cognitive decline associated with DM Type II occurs somewhat later with disease progression. Likewise, neurophysiological changes, including periventricular hyperintensities (Van Harten et al., 2007), white matter lesions (Manschot et al., 2006) as well as cortical and subcortical atrophy (Manschot et al., 2006) have been associated with reductions in motor speed and overall cognitive dysfunctioning in patients with DM Type II. While Van Harten et al. (2007), found no relation to memory performance but other cognitive domains, Manschot et al. (2006) identified decrements in areas of attention and executive functioning, information processing speed and memory.

The absence of a direct effect of DM Type II on cognitive performance contradicts findings from cross-sectional studies that suggest that effect sizes consistently range between −.3 and −.6. All in all, we conclude that DM Type II can aggravate the course of cognitive decline, mostly pertaining to tasks with a psychomotor component and executive functioning. Moreover, DM Type II alters cognitive decline typically observed in aging after the disease has been present for a longer period of time. In the visual thinking subtest this change is more pronounced for individuals suffering from MCI/AD. It is important to highlight the effect of dropout on our results. 50% of individuals that were initially diagnosed with DM Type II did not follow up to T3, suggesting systematic drop-out in our sample. It is plausible to assume that if these individuals were included in our analysis effects of DM Type II would be enlarged.

From a clinical perspective DM Type II might serve as a model for other concomitant diseases such as hypertension which become more frequent as people age and present cardiovascular risk factors that are associated with neurophysiological changes to the brain. These are likely to interact and/or mediate each other and the exact mechanisms by which cerebrovascular changes occur and their association with cognitive deficits remain unclear. In clinical practice these conditions need to be considered when diagnosing MCI or AD. In this respect, it is notable that in our study DM Type II did not affect declarative memory, which is typically compromised in MCI and early AD, but cognitive flexibility and visual thinking both of which contribute to executive functioning. The latter drives a number of other neuropsychological functions and is therefore crucial for neural compensation (Buschkühl et al., 2014). In addition, cognitive deficits are associated with severe functional disabilities in everyday life. Taken together, these

Table 2
Results of the repeated measures analyses.

Test	Factor	F, p
Word list	Time[a]	F(2,470)=0.31, p=.735
	DM Type II	F(2,235)=0.35, p=.704
	Cognitive status[b]	F(1,235)=2.57, p=.110
	DM Type II* cognitive status	F(2,235)=1.25, p=.287
	Time[1]*DM Type II	F(4,470)=0.59, p=.673
	Time[1]*DM Type II* cognitive status[b]	F(4,470)=0.75, p=.556
Digit symbol test	Time[a]	F(2,468)=2.76, p=.064
	DM Type II	F(2,234)=1.88, p=.156
	Cognitive status[b]	F(1,234)=20.80, p < .0001
	DM Type II* cognitive status	F(2,234)=0.07, p=.936
	Time[1]*DM Type II	F(4,468)=3.23, p=.012
	Time[1]*DM Type II* cognitive status[b]	F(4,468)=0.64, p=.872
Mosaic test	Time[a]	F(2,470)=1.88, p=.155
	DM Type II	F(2,235)=0.34, p=.713
	Cognitive status[b]	F(1,235)=8.01, p=.005
	DM Type II* cognitive status	F(2,235)=0.07,p=.937
	Time[1]*DM Type II	F(4,470)=1.05, p=.379
	Time[1]*DM Type II* cognitive status[b]	F(4,470)=0.97, p=.425
Finding similarities	Time[a]	F(2,472)=4.27, p=.014
	DM Type II	F(2,236)=0.02, p=.979
	Cognitive Status[b]	F(1,236)=12.59, p=.0005
	DM Type II*Cognitive Status	F(2,236)=0.69, p=.501
	Time[1]*DM Type II	F(4,472)=0.12, p=.975
	Time[1]*DM Type II* Cognitive Status[b]	F(4,472)=0.40, p=.809
Word fluency	Time[a]	F(2,466)=0.56, p=.569
	DM Type II	F(2,233)=0.76, p=.470
	Cognitive Status[b]	F(1,233)=6.15, p=.014
	DM Type II *Cognitive Status	F(2.234)=0.90, p=.410
	Time[1]*DM Type II	F(4,466)=1.36, p=.245
	Time[1]*DM Type II* Cognitive Status[b]	F(4,466)=1.20, p=.309
Visual thinking	Time[a]	F(2,464)=5.80, p=.003
	DM Type II	F(2,232)=0.27, p=.766
	Cognitive status[b]	F(1,232)=7.53, p=.007
	DM Type II* cognitive status	F(2,232)=0.14, p=.867
	Time[1]*DM Type II	F(4,464)=2.81, p=.025
	Time[1]*DM Type II* cognitive status[b]	F(4,464)=2.76, p=.027
D2 (Attention endurance)	Time[a]	F(2,446)=4.47, p=.012
	DM Type II	F(2,223)=0.44, p=.644
	Cognitive status[b]	F(1,223)=24.92, p < .0001
	DM Type II* cognitive status	F(2,223)=1.01, p=0.366
	Time[1]*DM Type II	F(4446)=0.34, p=.851
	Time[1]*DM Type II* cognitive status[b]	F(4446)=0.21, p=.935

[a] Time between examination waves (within subject factor in the repeated measures design).
[b] Diagnosis of MCI/AD or healthy control.

findings facilitate the hypothesis that concomitant diseases in general and DM Type II in particular can aggravate the course of MCI and early AD by aggravating neuropsychological deficits but do not have a direct effect on the pathophysiological changes involved.

The relatively long follow up interval of 14 years represents a clear strength of this study allowing for more accurate assessment

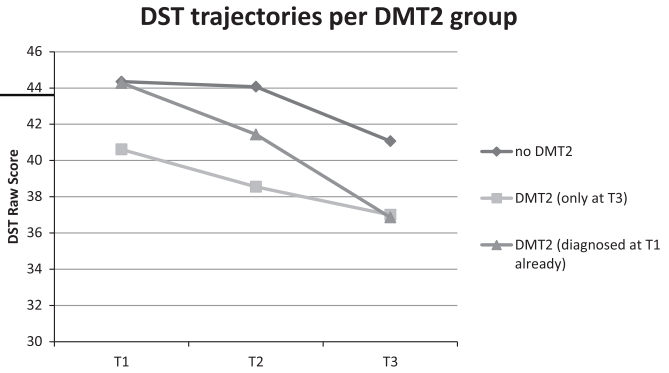

Fig. 2. Raw Scores for Digit-Symbol Test across examination waves.

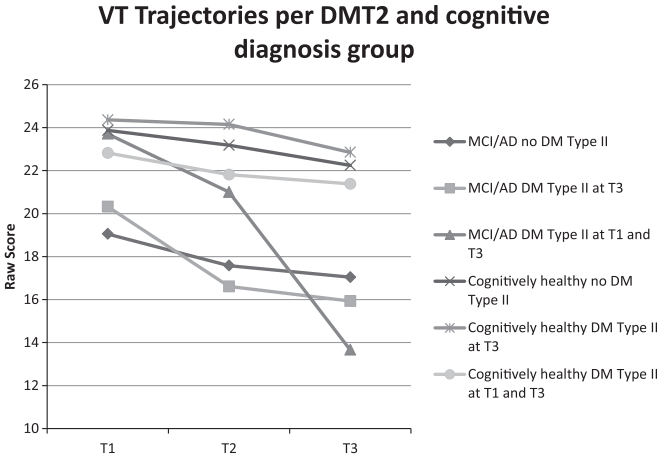

Fig. 3. Raw Scores for Visual Thinking across examination waves.

of the longitudinal dimensions of the impact of DM Type II on cognitive functioning. A potential drawback to this study is the absence of reliable records of when DM Type II was actually present. Recent estimates demonstrate that as many as half of all individuals suffering from DM Type II are undiagnosed. In this study, we mitigated this effect partially by classifying individuals according to our examinations, including DM Type II diagnostics at T1.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.psychres.2016.04.009.

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