GENEESKUNDE

master in de biomedische wetenschappen: klinische moleculaire wetenschappen

2010
2011

Masterproef

Effect of aging and genetic variations on decision making, fine motor and cognitive skills

Promotor :
Prof. dr. Raf MEESEN
Prof. dr. Niels HELLINGS

Lise Bogaers

Masterproef voorgedragen tot het bekomen van de graad van master in de biomedische wetenschappen , afstudeerrichting klinische moleculaire wetenschappen

Maastricht University

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UNIVERSITEIT VAN DE TOEKOMST

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Acknowledgements

This paper is the result of my senior practical training at the rehabilitation research centre (REVAL) and the Biomedical Research Institute (BIOMED). Over the past eight months I participated in a study that investigated the effect of aging and genetic variants on executive functioning, fine hand motor control and cognition. During this internship, I realized that research is not something you do on your own. Therefore, I would like to show my gratitude to everyone who assisted me and guided me during this internship.

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Lise Bogaers
June 13th, 2011

Abbreviations

ACC	Accuracy
BDNF	Brain-derived neurotrophic factor
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
DA	Dopamine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ER	Endoplasmic reticulum
LTP	Long-term potentiation
mBDNF	Mature brain-derived neurotrophic factor
Met	Methionine
MoCA	Montreal Cognitive Assessment
PCR	Polymerase chain reaction
PFC	Prefrontal cortex
PP	Purdue Pegboard
PPR hand	Purdue Pegboard right hand
PPL hand	Purdue Pegboard left
PPB hands	Purdue Pegboard both hands
SNP	Single nucleotide polymorphism
Val	Valine
WCS	Wisconsin Card Sorting

Samenvatting

Achtergrond en doelstelling: Het verouderingsproces gaat gepaard met cognitieve problemen, evenals verminderde motorische mogelijkheden. Meerdere *single nucleotide polymorphisms* werden reeds geassocieerd met neurale en cognitieve variaties in gezonde volwassenen. Bovendien wordt gesuggereerd dat de impact van genetische varianten verhoogd met leeftijd. In deze studie werd de impact van veroudering en genetische varianten op executieve functies, fijne hand motoriek en cognitieve vaardigheden onderzocht. Twee veel voorkomende Val/Met polymorfismen werden geëvalueerd. Met name, het COMT Val/Met polymorfisme heeft een invloed op catechol-O-methyltransferase, een enzym dat betrokken is bij de degradatie van dopamine in de prefrontale cortex. Het BDNF Val/Met polymorfisme beïnvloedt de *brain-derived neurotrophic factor*.

Methoden: Zevenenvijftig gezonde vrijwilligers namen deel aan de studie. De testpersonen werden onderverdeeld in twee leeftijdsgroepen: de jonge leeftijdsgroep (n = 27, leeftijd varieerde tussen 18 en 59 jaar) en de oudere leeftijdsgroep (n = 30, leeftijd varieerde van 60 tot 80 jaar). Genotypen werden bepaald na afname van wangslijmvliescel stalen. Executieve functies, in het bijzonder besluitvorming, werden bepaald door middel van de *local/global* taak. De *Purdue Pegboard* test werd afgenomen om fijne hand motoriek te evalueren. De oudere leeftijdsgroep werd ook nog getest voor milde cognitieve stoornissen aan de hand van de *Montreal Cognitive Assessment (MoCA)* test.

Resultaten: Statistisch analyse toonde aan dat jongeren significant beter presteerden op de taken die executieve functies en fijne hand motoriek evalueerden dan ouderen. Bovendien werd een grotere variabiliteit in prestatie scores waargenomen in de oudere groep. Alle ouderen scoorden voldoende punten op de *MoCA* test. Verder werd er geen effect van de verschillende BDNF en COMT genotypen op executieve functies, fijne hand motoriek en globale cognitie gevonden in gezonde volwassenen en ouderen. Daarenboven werd er geen COMT x BDNF interactie ontdekt.

Conclusie: De resultaten van deze studie tonen aan dat cognitieve en motorische processen afnemen naargelang men ouder wordt. Hoewel een grote variabiliteit bestaat bij ouderen, hebben de BDNF en COMT genotypen geen significant effect op prestaties van de geselecteerde taken. Gezien de geringe statistische power van deze studie is het noodzakelijk verder onderzoek te verrichten naar de bijdrage van genetische varianten aan cognitieve en motorische functies.

Abstract

Background and purpose: Aging is associated with a broad range of psychological and physiological changes, including a decline in cognition and in motor function. Several single nucleotide polymorphisms (SNPs) have been linked to neural and cognitive variation in healthy adults. Moreover, it is suggested that the effects of genetic variants are enhanced with human aging. The present study investigates whether aging and genetic variants influence executive functioning, fine hand motor control and cognitive skills. Two common Val/Met polymorphisms, one affecting the catechol-O-methyltransferase (COMT) enzyme, which degrades dopamine (DA) in the prefrontal cortex (PFC), and the other influencing the brain-derived neurotrophic factor (BDNF) were assessed.

Methods: The participants were fifty-seven healthy volunteers. Subjects were divided into two age groups. The young age group (n = 27) ranged from 18 to 59 years and the old age group (n = 30) ranged from 60 to 80 years. Genotypes were determined from buccal cell samples. Executive functioning, particularly decision making, was assessed by means of the local/global task. The Purdue Pegboard (PP) task was used to measure fine hand movements. To control for mild cognitive impairments, the elderly completed the Montreal Cognitive Assessment (MoCA) test.

Results: A Mann-Whitney U-test revealed that the younger age group performed significantly better on tasks evaluating executive functioning and fine hand motor skills compared to older people. Moreover, a great variability was seen among elderly. All elderly scored sufficient on the MoCA, which indicated that none of them was cognitive impaired. Furthermore, we did not observe any impact of the BDNF or COMT genotype on executive functioning, fine hand motor control and general cognition in healthy adults and healthy elderly. In addition, no COMT x BDNF interaction was revealed.

Conclusion: The findings of this study indicate that cognitive and motor processes decline with human aging. Although a great variability exist among elderly, the BDNF or COMT genotypes did not significantly influenced performance on the selected tasks. Given the low statistical power of the study, further research is still necessary to investigate to what extent genetic variants contribute to cognitive and motor functions.

1 Introduction

The population aging phenomenon is one of the most striking features of the last century. Due to longer life expectancy and decreased birth rate, the number and the proportion of older people (that is, those aged 60 years and over) in the society has increased[1]. Population ageing is enduring. Since 1950, the proportion of older persons has been rising steadily, passing from 8% in 1950 to 11% in 2009, and is expected to reach 22% in 2050[2]. As a consequence, the quality of life of elderly people has become relevant with the demographic shift towards an aging society[3]. The aging process in humans is associated with cognitive and physical inconveniences, which interfere with daily routines. Although each individual is different, abilities coupled to cognition, such as speed of processing, working memory and long-term memory tend to decline in old age[4-6]. In parallel, loss of motor function and slowing of motor movements is a common consequence of aging[7, 8]. Cousins et al. (1998) and other research groups have reported that finger tapping frequency lowers with advancing age [9,10,11]. Furthermore, Smith et al. (1999) have demonstrated a decline in fine motor hand movements in older people[12].

The underlying mechanism of aging is not completely understood, but the most obvious explanation for aging is the occurrence of structural and chemical changes in the brain[13]. As for normal aging, it was discovered that multiple brain structures show volumetric shrinkage and a decline in white matter integrity[14]. The most substantial shrinkage is observed in the prefrontal cortex (PFC), where the evidence of an association between volume shrinkage and aging deficits in executive and working memory function is more consistently found[15, 16]. Parallel to the less severe neuroanatomical changes, milder cognitive declines occurring during normal aging are more likely to be due to chemical shifts in still relatively intact neural circuitries, affecting the efficacy of neural information transfer[17]. To compensate for these age-related structural and neurochemical changes, reorganization and redistribution of functional networks was discovered in elderly. Results of several studies have shown that elderly subjects recruit additional cortical and subcortical areas even for the performance of a simple motor task[18, 19].

Individual differences in cognitive performance increase from early to late adulthood, likely reflecting influences of multiple factors, including different life experiences, genetic influences and susceptibility to neuropathology. In this senior internship, the influence of aging and genetics on executive functioning, fine motor hand control and cognitive skills are particularly of interest.

At present, close to 100 candidate genes affecting human brain functions and cognition have been reported[20]. For instance, the brain-derived neurotrophic factor (BDNF) gene, involved in neurotrophic systems and the catechol-O-methyltransferase (COMT) gene, implicated in dopaminergic systems have received much attention[21, 22].

1.1 The brain-derived neurotrophic factor polymorphism

The BDNF molecule is one of a family of neurotrophins that is implicated in almost all aspects of central nervous system (CNS) development, including neuronal proliferation and survival, synapse formation and synaptic plasticity[23]. Both BDNF and its tyrosine kinase receptor are widely distributed throughout the brain, with highest expression in the hippocampus [24]. BDNF is initially synthesized as a pre-proBDNF protein, which has its pre-sequence cleaved off in the endoplasmic reticulum (ER) (**figure 1**). The resulting proBDNF then transits to the Golgi apparatus. Here, proBDNF binds to intracellular sortilin to facilitate proper folding of the mature domain. Next, a motif in the mature domain of BDNF binds to carboxypeptidase E (CPE), an interaction that sorts BDNF into large dense core vesicles, which are a component of the regulated, activity-dependent secretory pathway. In the absence of this motif, BDNF is sorted into the constitutive pathway. Subsequently, proBDNF packaged in both types of vesicles is either proteolytically cleaved and secreted as mature BDNF (mBDNF), or secreted as proBDNF and cleaved by extracellular proteases. Both proBDNF and mBDNF are preferentially packaged into vesicles of the regulated secretory pathway and released from typical neurons[25-27].

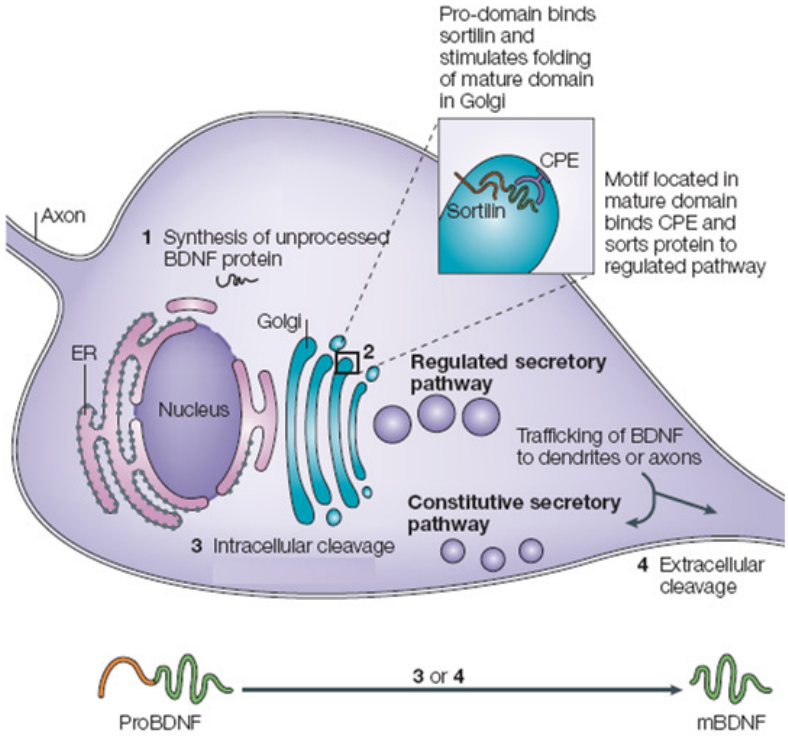

Figure 1. BDNF synthesis, processing, sorting, transport and secretion in neurons. BDNF is initially synthesized as a precursor protein (pre-proBDNF) in the ER. Following cleavage of the signal peptide, proBDNF is transported into the Golgi apparatus (1). To facilitate the correct folding of the mature domain, proBDNF binds to intracellular sortilin. In the appropriate conformation, the mature domain of BDNF binds to CPE, thereby sorting the neurotrophin to the regulated secretory pathway (2). In the absence of this motif, BDNF is sorted into the constitutive pathway. ProBDNF packaged in both types of vesicles is either proteolytically cleaved and secreted as mBDNF (3), or secreted as proBDNF and cleaved by extracellular proteases (4). Both proBDNF and mBDNF are preferentially packaged into vesicles of the regulated secretory pathway and released from neurons. BDNF, brain-derived neurotrophic factor; ER, endoplasmic reticulum; CPE, carboxypeptidase E; mBDNF, mature brain-derived neurotrophic factor. [Adapted from Lu et al, 2005]

Once released, BDNF activates different intracellular secondary messenger cascades which modulate synaptic plasticity, the ability of chemical synapses to change their strength. Long-lasting enhancement in signal transmission between two neurons, termed as long-term potentiation (LTP), is one of several mechanisms underlying synaptic plasticity[28]. As memories are thought to be encoded by modification of synaptic strength, LTP is widely regarded as one of the major cellular mechanisms that underlies learning, memory formation and sensorimotor recovery in nervous systems [28-30]. Several *in vitro* experiments have shown that BDNF is a key regulator of synaptic transmission and plasticity. For instance, Lohof et al. (1993) have

demonstrated that BDNF rapidly enhances synaptic transmission at the developing neuromuscular synapse in culture[31]. Another study, using hippocampal slices from young adult rats, has shown that the application of exogenous BDNF promotes the induction of rapid and long-lasting enhancement of synaptic strength[32]. *In vivo* experiments have demonstrated that mice lacking the BDNF receptor show abnormal hippocampal LTP and impaired learning behavior[33].

A single nucleotide polymorphism (SNP) in the human BDNF gene, resulting in a valine (Val) to methionine (Met) substitution at amino acid position 66 (Val66Met) in the pro-region of BDNF, has been identified[34]. This variant is relatively common: approximately 30-50% of people worldwide are heterozygous (Val/Met) for the Met substitution[35]. *In vitro* studies have provided evidence that the Met substitution is associated with abnormal intracellular trafficking and decreased activity-dependent secretion of the BDNF molecule[34, 36]. Measurement of BDNF levels in $BDNF^{Met/Met}$ mice revealed approximately a 30% reduction in BDNF activity-dependent release[37]. These findings indicate that the BDNF prodomain plays an important role in regulating their intracellular trafficking to secretory pathways.

1.1.1 Behavioral consequences of the BDNF polymorphism

In physiological conditions, the BDNF polymorphism (Val66Met) is associated with decreased hippocampal and PFC volumes[24, 38]. In healthy adults, a role for BDNF in memory and learning is supported by the finding that carriers of the Met allele (Val/Met and Met/Met) perform worse on tasks that involved recalling places and events (long-term episodic memory), but did not differ from Val/Val individuals on tasks that have been classically shown to be less hippocampal dependent, such as word learning and planning tasks (short-term memory)[34, 39]. In contrast to the results of Egan et al. (2003), a number of studies have reported that the Met allele reduces also short-term memory[40] and general intelligence[41]. Several animal studies have shown that the beneficial effects of the BDNF molecule on neural plasticity may be reduced with age[42, 43]. This finding implies that older BDNF Met carriers are especially vulnerable to cognitive declines. Indeed, Miyajima et al. (2008) reported that in non-demented elderly the Met allele is associated with a greater global cognitive decline as compared to Val/Val homozygotes[44]. On the other hand, a study of Harris et al. (2006) indicates that older Met homozygotes have greater reasoning

skills than Val homozygotes, suggesting a protective effect for the Met allele at older age[45]. Besides its influence on cognition, the BDNF polymorphism is also associated with differences in brain motor system function and motor learning. In a study of McHughen et al. (2010) functional magnetic resonance imaging scanning during right index finger movement identified activation in a broad sensorimotor network in healthy adults. However, subjects with the polymorphism showed smaller activation volume within several brain regions as compared with subjects without the polymorphism. Furthermore, it was demonstrated that Val/Met subjects showed greater error during short-term learning and poorer retention over 4 days, relative to subjects without the polymorphism[38]. In addition, Fritsch and colleagues (2010) have shown that learning a particular motor task is BDNF genotype dependent. While both groups started off with comparable baseline performance, Met carriers displayed significantly reduced motor skill acquisition by the end of the training session (day 5) relative to Val/Val adults[46]. To date, to our knowledge, no studies investigated the association between the BDNF genotype and fine hand motor control in healthy elderly.

1.2 The catechol-O-methyltransferase polymorphism

The second SNP that may be highly relevant to cognitive aging is that one of the COMT gene. COMT enzymatic activity results in the degradation of dopamine (DA), particularly in the PFC[47]. A functional polymorphism (common normal variant) of the COMT gene is characterized by a substitution of methionine (Met) in place of valine (Val) at codon 158 (Val158Met) and renders the enzyme thermolabile, thereby leading to reduced COMT enzymatic activity at body temperature[48]. Presence of the Val allele results in a 4-fold increase in COMT enzymatic activity. As a consequence, DA degradation is increased and dopaminergic stimulation of the post-synaptic neuron is reduced[49]. Lower enzymatic activity among Met carriers leads to less frontal DA degradation and hence greater DA availability at the DA receptors[50, 51].

Several studies have provided basic evidence of an influence of COMT on cognition. Both animal and human research have demonstrated an enhancement in working memory performance after the administration of COMT inhibitors[52, 53].

1.2.1 Behavioral consequences of the COMT polymorphism

Thus far, most studies investigating COMT effects have involved normal younger adults and schizophrenic patients. In these populations, the Met/Met form of the COMT polymorphism has been related to superior performance in executive functioning (i.e. fewer errors in the Wisconsin Card Sorting task)[50, 54, 55] as well as in speed and attention[56, 57]. Furthermore, some studies have investigated this relationship in older adults, a group known to experience impairments in prefrontal cognitive functions. De Frias and colleagues (2005) found that compared to Val carriers overall, Met/Met individuals performed better on tests measuring executive functions, visuospatial skill and episodic and semantic memory[58]. On the other hand, Harris et al. (2005) found that among healthy older adults Val/Met heterozygotes had higher scores on measures of episodic and semantic memory than both homozygous groups[59]. Yet others have reported an advantage of Met/Met and Val/Met carriers on tasks assessing speed of information processing[60]. Only one study found no association between the COMT polymorphism and cognitive measures in elderly[61].

Above mentioned findings led to the suggestion that the COMT polymorphism may exert effects on cognition by modulating prefrontal DA function. Animal and human data suggest that the relation between DA levels and cognitive functioning follows an inverted U-shaped curve (**figure 2**), with both suboptimal and supra-optimal dopamine activity impairing cognitive performance[62, 63]. Consistent with data implicating an inverted U-response function, pharmacological studies in animals[64] and in healthy humans[65, 66] indicate that the effect of dopamimetic agents on the PFC depends on the baseline level of PFC function, which is presumably a reflection of one's position on the putative inverted U-curve. Indeed, in healthy subjects, relatively poor performers on prefrontal cognitive tasks tend to improve after administration of dopamimetic agents, whereas high performers show no response or get worse[65, 66].

An individual's location on this hypothesized inverted U-curve is likely to be determined by factors influencing baseline prefrontal dopamine level, including age and COMT genotype[67, 68]. At molecular level, studies indicate that the age-related dopaminergic function losses[69, 70] are strong mediators of age-related deficits in multiple cognitive tasks[71]. Given the relation between age-related dopaminergic losses and age-related cognitive decline, it is plausible to assume that advancing age shifts individuals toward the left side of the curve relating DA signaling to cognitive performance, that is, further away from the optimal DA level. Because Val carriers of

the COMT gene have relatively low DA levels, this leftward shift is more pronounced in Val/Val homozygotes. Thus, it is predicted that human aging magnifies the effect of the COMT polymorphism on cognitive performance[15, 22, 60, 67, 68]. In a recent study, a significant COMT × age interaction was indeed observed, with Met homozygosity being associated with improved executive performance of older but not younger adults[22].

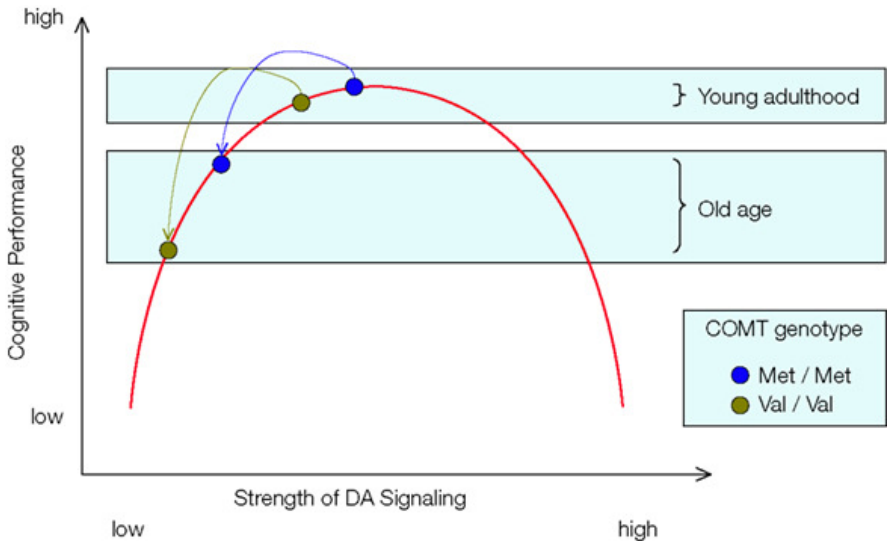

Figure 2. Inverted U-shaped function linking the strength of DA signaling to cognitive performance. Cognitive function is optimal when dopamine activity is neither too low nor too high, corresponding to the top of the curve. Performance differences between Val and Met carriers are greater in elderly, reflecting the decrease in dopaminergic neuromodulation with advancing age. DA, dopamine; Val, Valine; Met, Methionine. [Linderberger et al, 2008]

Data about the effects of the COMT polymorphism on motor function are scarce. Holtzer et al. (2010) investigated the association between the Val158Met polymorphism and gait velocity in older adults. It was demonstrated that Met/Val heterozygotes are associated with faster gait velocity as compared to both homozygous forms[72]. In addition, several studies suggest an association between DA levels and motor learning. Results obtained with positron emission tomography in humans demonstrate that during finger sequence learning DA is increased in the supplementary motor area[73]. This study suggests that dopaminergic projections to motor cortical areas play a role in movement learning. Other research groups have demonstrated an enhancement of motor learning by systematic application of levodopa (DA precursor) in healthy individuals[74, 75] and stroke patients[76, 77]. However, systematic application affects dopaminergic transmission throughout the whole brain and therefore is not specific for motor function. To our

knowledge, associations between the Val158Met COMT polymorphism and fine motor hand control in healthy elderly have not been reported yet.

1.3 Interactional effects of COMT and BDNF genotypes

Recent publications stress the need to consider gene-gene interactions. Studies on gene-gene interactions involving COMT have focused on interdependencies between the COMT gene and other genes linked the dopaminergic system, such as the DA transporter gene[77] and the DA receptor genes[78]. In contrast, Nagel et al. (2008) investigated the interaction between the COMT gene and the BDNF gene and demonstrated that older adults carrying two COMT Val alleles and at least one BDNF Met allele were particularly slow in performing the Wisconsin Card Sorting (WSC) task, a measure of executive functioning[22]. Both genes play a pronounced role in the PFC. Furthermore, BDNF influences DA release in striatal regions, which may interact with COMT effects on prefrontal DA degradation[79]. Thus, for several reasons the COMT and BDNF genes may interactively influence cognitive functioning. To our knowledge there are no studies investigating the link between gene-gene interactions and fine motor control. Therefore, in this study we will also focus on this link.

1.4 Study outline

As the proportion of older people in societies has increased, research into the determinants of aging has risen in importance. The aim of this study was to examine the effect of aging and genetic variations on executive functioning (decision making, inhibition and working memory), fine motor hand control and cognitive skills. First, we compared performance differences with respect to executive functions and fine hand movements between younger and older subjects. Second, the influence of two genetic variants (COMT Val158Met and BDNF Val66Met), with respect of their individual and shared contribution to executive functioning, fine motor hand control, and cognitive ability were analyzed for the whole subject sample. In addition, it was examined whether human aging magnifies the consequences of genetic variation. To achieve these objectives, healthy adults and healthy elderly were genotyped for both the COMT and BDNF genes and all subjects completed a test assessing executive functioning and a fine hand

motor task. Additionally, the elderly finished a global cognition test, involving different cognitive domains.

2 Materials and methods

2.1 Subjects

Fifty-seven healthy volunteers participated to the present study. Subjects were divided into two age groups. The young age group (n = 27) ranged from 18 to 59 years (mean age = 44.98, SD = 12.53, 14 women and 13 men). The old age group (n = 30) ranged from 60 to 80 years (mean age = 70.45, SD = 5.94, 16 women and 14 men). According to the Oldfield questionnaire[80] all subjects are right-hand dominant (Oldfield score: mean = 97.30, SD = 9.42). Visual and auditory acuity for cognitive and fine motor testing was sufficient for all participants. Exclusion criteria were: movement disorders of the upper limbs, a history of medical, neurological or psychiatric disease, and severe alcohol or drug abuse. All subjects gave informed consent according to the protocol approved by the local research Ethical Committee of the University of Hasselt.

2.2 Genetic analysis

In the public SNP database (http://www.ncbi.nlm.nih.gov/SNP), a common coding variant in the human BDNF gene (rs6265) was identified, namely a guanine (G) which has been replaced by an adenine (A). This polymorphism is responsible for a Val66Met change at the amino acid level. In addition, a common polymorphism in the COMT gene (rs4680) was analyzed. Here, a G to an A transition at codon 158 resulted in a Val to Met amino acid substitution. In order to establish both COMT and BDNF genotypes, two buccal swabs (M10022, MLS, Menen, Belgium) were obtained from all subjects and genomic deoxyribonucleic acid (DNA) was extracted. Both genotypes were characterized by polymerase chain reaction (PCR) and DNA sequencing.

2.2.1 DNA extraction

DNA was extracted from buccal swabs using the Chelex 100 Resin (143-2832, Bio-Rad, Nazareth Eke, Belgium) extraction method (supplement 1). Total DNA yield and purity were measured by UV absorbance at 260 nm (ND-1000, Thermo Scientific, Westburg, Leusden).

2.2.2 BDNF genotyping

The BDNF gene was amplified using nested PCR. The following forward primer 5'-AAAGAAGCAAACATCCGAGGACAAG-3' and reverse primer 5'-ATTCCTCCAGCAGAAAGAGAAGAGG-3' were used. The PCR fragments were expected to result in a 274 base pair (bp) product. A MyCycler thermal cycler (Bio-Rad Laboratories, Nazareth Eke, Belgium) was used for DNA amplification. The first amplification reactions were performed in a total volume of 50 µL, containing approximately 50 ng of genomic DNA as template, 10 µM of each primer (custom-made, Eurogentec, Liege, Belgium), 200 µM deoxyribonucleotide triphospates (dNTP), 5x High Fidelity Buffer inclusive of magnesium chloride ($MgCl_2$), 1 U *Taq* polymerase (Verbatim High Fidelity PCR Kit, AB-1920/A/N, Thermo Fisher Scientific, Westburg, Leusden) and autoclaved MilliQ (MQ). The PCR cycling conditions consisted of an initial denaturation step for 4 min at 94°C, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 45 s, followed by a final extension at 72°C for 10 min and cooling to 4°C. After Sephadex™ G-50 (17-0043-02, GE Healthcare Bio-Sciences, Diegem, Belgium) purification, the PCR products were used as a template in de second PCR round. The second amplification reactions were performed under the same conditions as the first amplification reactions, except DNA template is 150 ng and PCR cycle numbers were reduced to 25. After nested PCR, samples were loaded on a 1,5% agarose gel (agarose powder, 1x Tris-acetate-EDTA (TAE) buffer and ethidium bromide) using Orange G (195235, Sigma-Aldrich). The size of the PCR fragments was determined using a 100 bp DNA ladder (15628-050, Invitrogen, Carlsbad, USA). A current of 130 volt was applied for 45 min, after which DNA was made visible under ultraviolet (UV) light, and a picture of the gel was taken using the Universal Hood imaging system (Bio-Rad laboratories, Milan, Italy). Furthermore, DNA sequencing was carried out using the BigDye® Terminator v1.1 Cycle Sequencing Kit (4337452, Applied Biosystems, Warrington, United Kingdom), the sequence primer 5'-AAAGAAGCAAACATCCGAGGACAAG-3' and 5-20 ng of PCR product. The sequencing extension products were purified utilizing Sephadex™, evaporated, and dissolved in highly deionized formamide (Hi-Di™ Formamide, 4311320, Applied Biosystems, Warrington, United Kingdom). The purified products were analyzed using an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems). DNA sequencing results were analyzed by means of Chromas 2.13 (Technelysium). Hardy-Weinberg equilibrium for genotype distribution was assessed.

2.2.3 COMT genotyping

The COMT gene was amplified using PCR. The following forward primer 5'-GGGCCTACTGTGGCTACTCA-3' and reverse primer 5'- GGCCCTTTTTCCAGGTCTGACA -3' were used to amplify the 178 bp polymorphic COMT fragment. The amplification was done in 50 µL reactions containing 50 ng of genomic DNA, 10 µM of each primer (custom-made, Eurogentec, Liege, Belgium), 200 µM dNTP, 5x GC Buffer inclusive of $MgCl_2$, 1 U *Taq* polymerase (Verbatim High Fidelity PCR Kit) and MQ. Cycling conditions were as follows: initial denaturation was at 95°C for 5 min, followed by 25 temperature cycles consisting of 20 s at 98°C for denaturation, 15 s at 60°C for annealing and 30 s at 72°C for extension, followed by a final elongation at 72°C for 10 min and cooling to 4°C. Success of the PCR was evaluated on a 1,5% agarose gel to which a current of 130 volt was applied for 45 min. DNA was made visible under UV light in a Universal Hood imaging system. Next, the agarose band containing the sample of interest (178 bp) was cut out. DNA was purified using the GFX PCR DNA and Gel Band Purification Kit (28-9034-70, GE Healthcare, Freiburg, Germany) and subsequently used in the sequencing reaction. DNA sequencing was carried out using the BigDye® Terminator v1.1 Cycle Sequencing Kit, the sequence primer 5'-ACTGTGGCTACTCAGCTGTG-3' and 5-20 ng PCR product. After Sephadex™ purification, the sequencing extension products were evaporated, dissolved in highly deionized formamide, and analyzed using an ABI PRISM® 310 DNA Analyzer. DNA sequencing results were interpreted by means of Chromas 2.13. Hardy-Weinberg equilibrium for genotype distribution was assessed.

2.3 Cognitive measures

To test executive functioning, all subjects performed the local/global computer task. Furthermore, the older sample completed the Montreal Cognitive Assessment (MoCA) test, a screening instrument used to detect mild cognitive impairments.

2.3.1 Local/global task

The local/global task is developed at the Centre for Movement Control and Neuroplasticity (K.U.Leuven). It is a computer-administered, neuropsychological test to measure executive functions. Working memory, inhibition and particularly decision making are important processes to properly perform this test. The participants completed three subtests. During these 3 subtests (**figure 3**), subjects had to decide whether they had to look at the contour (global, i.e. a square or rectangle) or at the elements (local, i.e. squares or rectangles) of the figure (stimulus). If the cue was large, subjects had to look at the contour of the figure and if the cue was small, subjects had to look at the elements of the figure. If the answer was a square, participants responded by pressing the number 1 on the computer keyboard. If the answer was a rectangle, participants responded by pressing the number 2. In the first subtest (= block 1; 28 repeat trials) the cue was always large. In the second subtest (= block 2; 28 repeat trials) the cue was always small. Subtest 3 combined the previous subtests: the large and small cues were being alternated (= block 3; 57 trials: 33 repeat trials and 24 switch trials). Before each subtest, the participants had the opportunity to practice. Feedback was provided during practice but never during performance of the tests. A limited time (3 s) was given to the participants to answer. Participants were instructed to perform the test as fast and accurately as possible. The percentage of correct answers (= % accuracy, % ACC) were used to index performance. Test duration was approximately 30 minutes.

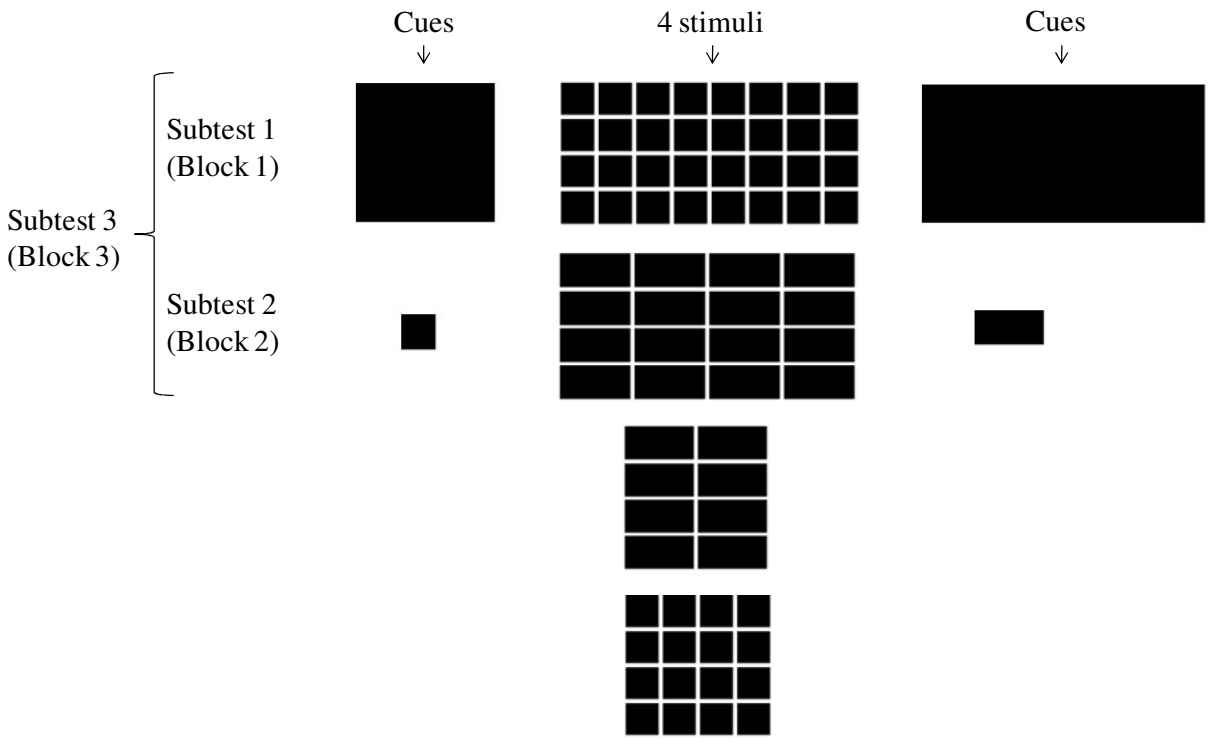

Figure 3. Visual representation of the computer-administered local/global task. One of the 4 different stimuli was projected in the middle of the computer screen: a large rectangle containing small squares, a large rectangle containing small rectangles, a large square containing small rectangles or a large square containing small squares. Two cues were projected at the sides of the computer screen. The cues could be either large or small. If the cues were large, participants had to look at the contour of the stimulus. If the cues were small, participants had to look at the elements of the stimulus. Subtest 1: cues are always large, which means that the participants had to look at the contour of the stimulus (28 trials). Subtest 2: cues are always small, thus participants had to look at the elements of the stimulus (28 trials). Subtest 3: cues were being alternated and could be either large or small (57 trials).

2.3.2 MoCA

The MoCA was designed as a screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. Time to administer the MoCA is approximately 10 minutes. A study of Thissen et al. (2010) reported that sensitivity and specificity of the Dutch version of the MoCA was sufficient to discriminate properly between healthy elderly and mild cognitive impaired elderly with a cut-off score of 20. The total possible score is 30 points[81].

Overview of all subtests:

- Visuospatial/executive: the subjects were instructed to draw a line, going from a number to a letter in ascending order, to copy a cube and to draw a clock with time set at ten past eleven.
- Naming: the participants were asked to name three animals on given pictures.
- Memory: the participants had to repeat five words after dictated by the examiner. The ability to memorize these words was tested by a delayed recall at the end of the cognitive test battery.
- Attention: in the Digit Span Forward task, participants were required to verbally repeat these digits in the order in which they were presented. In the Digit Span Backward task, participants were required to produce the digits in the reverse order of presentation. Next, the examiner read the list of letters at a rate of one per second. Every time the letter A was called, the subject had to tap his hand once. Furthermore, a mathematic test was performed. Subjects were asked to subtract seven from 100. Participants had to continue subtracting seven from the next answer until the number 65 was reached.
- Language: participants were instructed to repeat a sentence exactly and to generate aloud as many words as possible beginning with the letter D in 1 min.
- Abstraction: the subjects had to explain the similarity between two given words.
- Orientation: the subjects were asked to name the date, place and location.

For detailed administration and scoring instructions, see supplement 2.

2.4 Fine hand motor measure

The Purdue Pegboard (PP) task is a reliable and valid method to assess bilateral manual dexterity and fine motor hand function (Tiffin 1948[82]). The PP (Model 32020, Lafayette Instrument Co, Lafayette, Indiana) consists of 50 holes arranged in two parallel columns and pegs at the top of the board. The pegs were placed in cups on the far right and far left of the board and could be reached conveniently by both hands. Subjects were instructed to start the test on a verbal cue while an examiner timed the test with a stopwatch. The test consisted of three subsets. Subjects

had 30 seconds to fill the holes with pegs initially with the dominant (right) hand, then with the non-dominant (left) hand, and finally with both hands simultaneously. Each subset was repeated three times to obtain an average. The test scores equaled the number of filled holes.

2.5 Statistical analyses

The presence of Hardy-Weinberg equilibrium for the genotype distributions was examined using the chi-square test for goodness of fit. Normality of behavioral data (performance scores on local/global, PP and MoCA) was checked using the Shapiro-Wilk test. Because data were not normal distributed ($p < 0.05$), statistical analysis was performed using the non-parametric Mann-Whitney U-test or Wilcoxon Signed rank test. Effects were considered significant if p-values were less than 0.05. All significant data were represented in graphs and expressed as mean ± standard deviation (SD). Statistical tests were carried out using SPSS analytical software for Windows.

3 Results

3.1 DNA extraction from buccal cell swabs

DNA was prepared from epithelial cells of the internal portion of the cheek (buccal cells). First, the buccal cells were collected by the use of swabs. This collection method is fast, easy to perform, non-invasive and inexpensive. DNA was extracted from buccal swabs using the Chelex 100 Resin extraction method. DNA quantity and quality were assessed using the spectrophotometer NanoDrop. All samples showed a low DNA concentration (~ 3 ng/µL). The 260/280 ratio was ~ 1.5, whereas a ratio of ~1.8 is generally accepted as "pure" for DNA. In conclusion, quantity and purity of DNA obtained from buccal cells was low.

3.2 Optimization BDNF genotyping

First, a conventional PCR using the 'standard' DNA *Taq* polymerase (Roche, Mannheim, Germany) was performed to amplify the BDNF sequence of interest. Because amplification proceeded inefficiently, a nested PCR (existed out of two PCR runs) was performed. Nested PCR (second run) produced a clear 274 bp band (**figure 4**, lane 2 to 11) where the intermediate PCR (first run) was very weak or even invisible (data not shown). A negative control was loaded in lane 12. For all the subjects, nested PCR was successful.

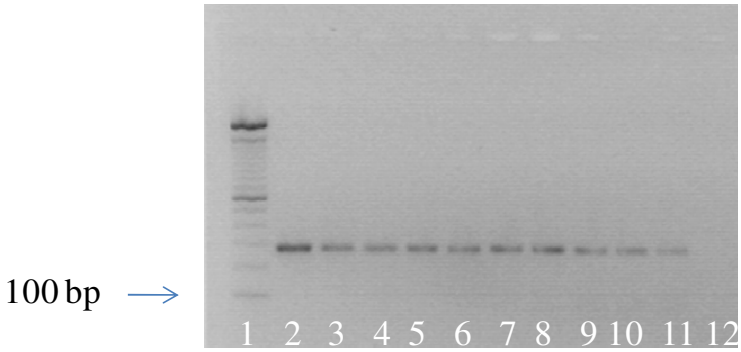

Figure 4. Agarose gel representing a 274 bp band of the BDNF gene of 10 subjects after a nested PCR. A 100 bp ladder was loaded on lane 1, subject samples were loaded on lane 2 to 11 and a negative control was loaded on lane 12. BDNF, brain-derived neurotrophic factor; PCR, polymerase chain reaction.

Next, all PCR fragments were sequenced. The automated DNA sequencer had generated a four-color chromatogram showing the results of the sequencing run, as well as a computer program's best guess at interpreting that data - a text file of sequence data. The 274 bp sequence of the BDNF gene was analyzed using Chromas 2.13 (**figure 5**). Individuals who were G/G homozygous (figure 5A) produced only the valine containing isoform of the BDNF protein. Here, the sequence chromatogram only showed a black (= G) peak at position 136. G/A Heterozygous individuals (figure 5B) produced both valine and methionine isoforms. Here, a black (= G) and a green (= A) peak were present at position 136, but at roughly half the height they would be if they were homozygous. In sum, fifty-five out of fifty-seven participants were successfully genotyped for the BDNF gene (success rate = 96.5%). The BDNF genotype distribution included 65% homozygotes for Val, 35% heterozygotes and 0% homozygotes for Met. Allele frequencies did not differ significantly from Hardy-Weinberg equilibrium (chi-square = 2.45, $p > 0.05$).

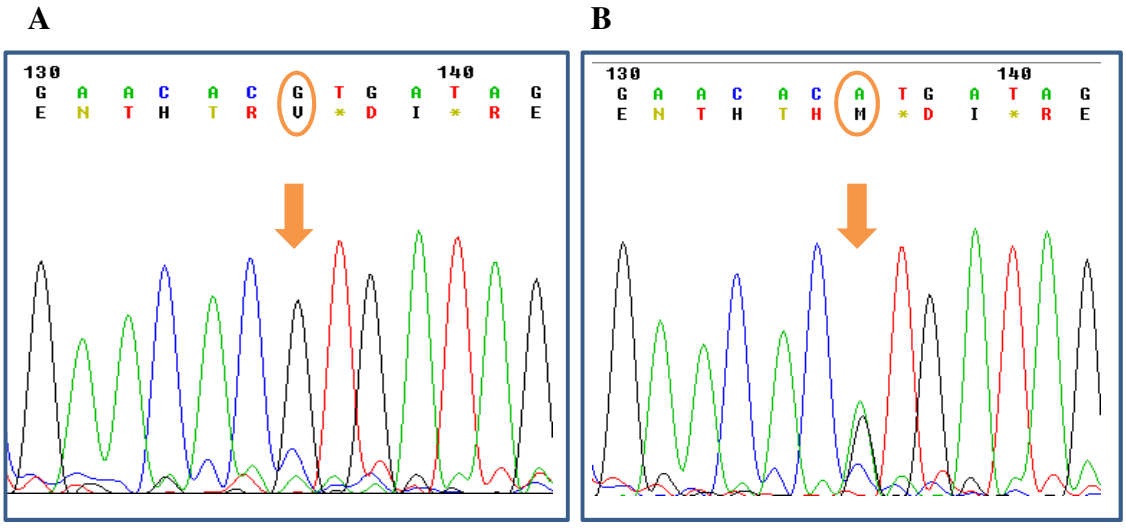

Figure 5. Sequencing chromatogram showing the part of interest of the 274 bp BDNF DNA sequence. A) Example of a G/G homozygous individual. Only a black peak (= G) was present at position 136 (arrow). The text DNA sequence confirmed the peak pattern by showing a G nucleotide and a V amino acid (circle) at position 136. B) Example of a G/A heterozygous individual. In this case, one allele carries a G, while the other has an A. Both peaks were present (black = G and green = A) at position 136 (arrow). The SNP is missed by the basecaller, because the text sequence simply shows an A nucleotide and an M amino acid (circle). BDNF, brain-derived neurotrophic factor; G, guanine; V, valine; A, adenine; M, methionine; SNP, single nucleotide polymorphism.

3.3 Optimization COMT genotyping

To amplify the genomic DNA fragment containing the COMT polymorphism (G to A transition) two primer pairs (**table 1** and **figure 6**) were tested.

Table 1. Primer pairs selected to amplify the genomic DNA fragment containing the COMT polymorphism.

Primer pair	Forward	Reverse
1	5'-ACTGTGGCTACTCAGCTGTG-3'	5'-CCTTTTTCCAGGTCTGACAA-3'
2	5'-GGGCCTACTGTGGCTACTCA-3'	5'-GGCCCTTTTTCCAGGTCTGACA-3'

A gradient PCR experiment with an annealing temperature range of 50°C to 66 °C was performed using 100 ng of DNA template of positive control samples (genomic DNA extracted from blood).

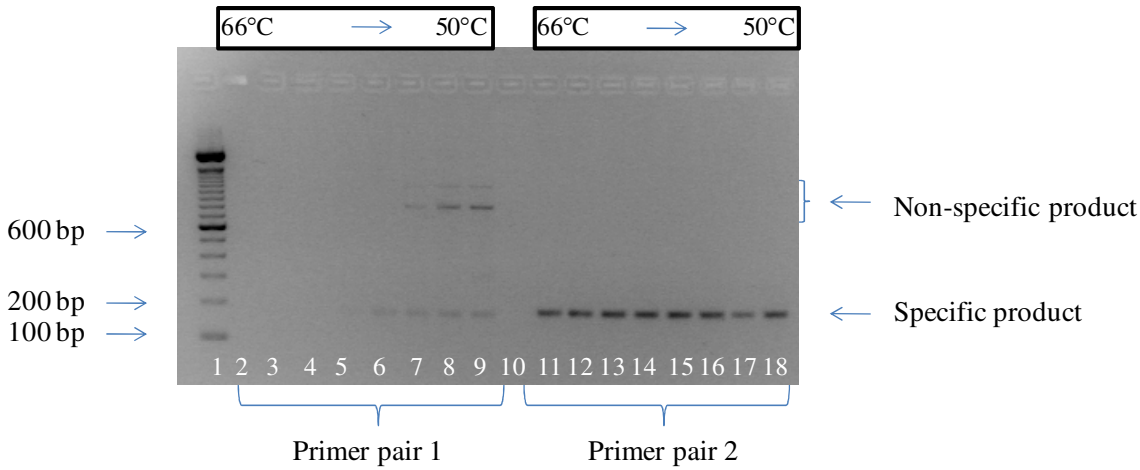

Figure 6. Agarose gel representing gradient PCR with temperatures from 66°C to 50°C to test two primer pairs. Lane 1: 100 bp ladder, lane 2-9: primer pair 1 and lane 11-18: primer pair 2. PCR, polymerase chain reaction.

The PCR fragments amplified using primer pair 1 and 2 were expected to result in a 169 bp and 178 bp product, respectively. **Figure 6** exhibited clearly that the temperatures between 66°C and 50°C work well for primer pair 2 (lane 11 to 18). In contrast, for primer pair 1, at temperatures between 53°C and 50°C a pattern of non-specific product amplification appeared (lane 7 to 9). At temperatures between 56°C and 50°C (lane 5 to 9), the specific product of 169 bp was visible, but the intensity of the products was low. At temperatures higher than 56°C, no product was present

(lane 2 to 4). In conclusion, primer pair 2 was selected to continue the COMT genotyping experiment using DNA extracted from buccal cell swabs. Subsequently, the DNA fragments containing the COMT polymorphism of the subjects were amplified using the 'standard' *Taq* polymerase. By using primer pair 2, PCR products were invisible (data not shown). Therefore, several methods were used to optimize PCR efficiency (**table 2**).

Table 2. PCR optimization methods and their corresponding effects.

PCR optimization method	**Effect**
Addition of 5% DMSO*	None
Increase in $MgCl_2$ concentration (up to 3 mM)	None
HotStar HiFidelity PCR Kit (with Q-solution)**	None
Verbatim High Fidelity PCR Kit	**Specific band of low intensity**
Nested PCR ***	Band smearing

* DMSO, Dimethyl sulfoxide

** 202602, Qiagen, Venlo, The Netherlands

*** by use of the Verbatim High Fidelity PCR Kit

By using the Verbatim High Fidelity PCR Kit, a specific but weak band of 178 bp appeared (**figure 7**; lane 3, 5, 6, 7, 8, 9, 10, 11 and 12). Unfortunately, also a light smear was visible in lane 5, 7, 9 and 11. PCR products in lane 2 and 4 were invisible. To exclude the non-specific (smear) PCR products and primer dimers (i.e. the lower band in lane 2 to 12), the agarose bands containing the 178 bp fragments were cut out.

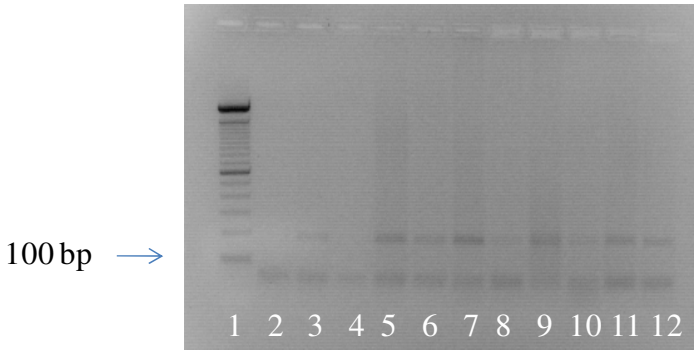

Figure 7. Agarose gel representing a 178 bp band of the COMT gene of 11 subjects after PCR using the Verbatim High Fidelity PCR kit. A 100 bp ladder was loaded on lane 1, subject samples were loaded on lane 2 to 12. COMT, catechol-O-methyltransferase; PCR, polymerase chain reaction.

DNA was purified from the agarose gel and sequenced using the forward or reverse primer of primer pair 2. Because all sequence reactions failed, the forward primer of primer pair 1 was used. Sequencing results are depicted in **figure 8**. Individuals who were G/G homozygous (figure 8A) produced only the valine containing isoform of the COMT enzyme. Here, the sequence chromatogram only showed a black (= G) peak at position 97. G/A Heterozygous individuals (figure 8B) produced both valine and methionine isoforms. Here, a black (= G) and a green (= A) peak were present at position 99, but at roughly half the height they would be if they were homozygous. Individuals who were A/A homozygous (figure 8C) produced only the methionine containing isoform of the COMT enzyme, which resulted in a one green (= A) peak at position 98 in the sequence chromatogram. Overall, seventeen out of twenty-four participants were successfully genotyped for the COMT gene (success rate = 70.8%). The COMT genotype distribution included 18% homozygotes for Val, 53% heterozygotes and 29% homozygotes for Met. All genotypes were consistent with proportions expected under Hardy-Weinberg equilibrium (chi-square = 0.09, $p > 0.05$).

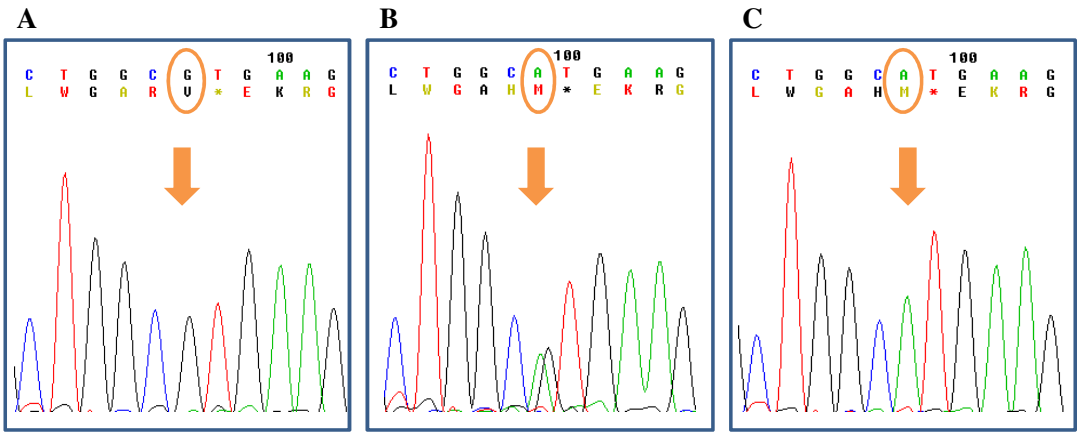

Figure 8. Sequencing chromatogram showing the part of interest of COMT DNA sequence. A) Example of a G/G homozygous individual. Only a black peak (= G) was present at position 97 (arrow). The text DNA sequence confirmed the peak pattern by showing a G nucleotide and a V amino acid (circle) at position 97. B) Example of a G/A heterozygous individual. In this case, one allele carries a G, while the other has an A. Both peaks were present (black = G and green = A) at position 99 (arrow). The SNP is missed by the basecaller, because the text sequence simply shows an A nucleotide and an M amino acid (circle). C) Example of a A/A homozygous individual. Only a green peak (= A) was present at position 98 (arrow). The text DNA sequence confirmed the peak pattern by showing an A nucleotide and an M amino acid (circle) at position 98. BDNF, brain-derived neurotrophic factor; G, guanine; V, valine; A, adenine; M, methionine; SNP, single nucleotide polymorphism.

3.4 Demographics

Characteristics of the study population are presented in **table 3**.

Table 3. Subject sample.

Variables	Young age group (age range 18-59)	Old age group (age range 60-80)
n	27	30
Mean age ± SD	44.98 ± 12.53	70.45 ± 5.94
Women/Men	14/13	16/14
BDNF genotype	26	29
• **Val/Val**	18	19
• **Val/Met**	8	10
• **Met/Met**	0	0
COMT genotype	8	8
• **Val/Val**	2	1
• **Val/Met**	3	5
• **Met/Met**	3	2

3.5 Behavioral consequences of aging

To investigate the effect of aging on executive functioning and fine hand motor control, regardless of the genotypes, the test sample was divided into two age groups: the young age group (18-59 years of age, $n = 27$) and the old age group (60-80 years of age, $n = 30$). According to the Mann-Whitney U-test the percentage of local/global accuracy (ACC) scores differed significant between the old age and the young age group (**figure 9**). The percentage of correct answers were significantly higher in the young age group compared to the old age group (average scores block 1+2: 81.15% vs. 80.77%, respectively, $p = 0.04$; average scores block 3: 73.41% vs. 61.94%, respectively, $p = 0.00$). However, for both age groups, average scores on block 1+2 were almost equal and standard deviations were small. Hence, the significant difference between the young and old age group for local/global block 1+2 seemed unrealistic. As a result, frequencies of % correct answers on block 1+2 were calculated (**table 4**). Striking was that many subjects achieved the same scores. For example, in the young age group, 21 out of 27 subjects scored

82.14%. In the old age group 15 out of 30 participants achieved a score of 82.14% and the other 15 older subject scored slightly different from 82.14%.

Table 4. Frequencies of % correct answers on block 1+2.

Age group	Scores block 1+2 (%)	Frequency
Young age group	66.07	1
	78.57	1
	80,36	4
	82,14	21
Old age group	76.79	3
	78.57	2
	80.36	10
	82.14	15

A Wilcoxon signed-rank test revealed that both age groups scored significantly better on block 1+2 (repeat trials, considered as 'easy') compared to block 3 (combination of repeat and switch trials, considered as 'difficult') (young age group, $p = 0.01$; old age group, $p = 0.00$; **figure 9**).

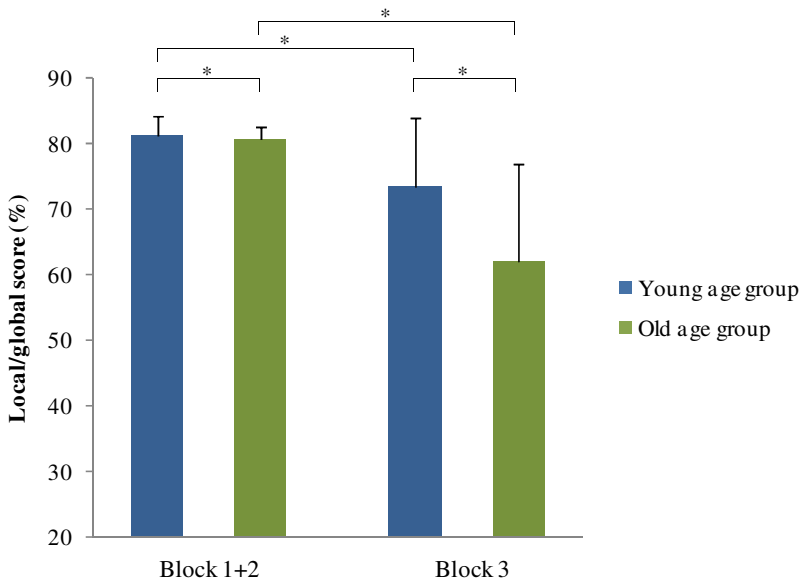

Figure 9. Graph representing the effect of aging on ACC scores (% correct answers) of the local/global task (mean ± SD). The percentage of correct answers was significantly higher in the young age group compared to the old age group for both block 1+2 and 3. Both age groups scored significantly better on block 1+2 compared to block 3. ACC, accuracy; * $p < 0.05$.

Furthermore, the Mann-Whitney U-test revealed a significant difference between the young age group and the old age group for the Purdue Pegboard right hand (PPR) score, Purdue Pegboard left hand (PPL) score and Purdue Pegboard both hands (PPB) score (all, $p < 0.05$; **figure 10**). More specific, the number of filled holes was significantly higher for the young age group compared to the old age group (average scores PPR: 14.58 vs. 12.31, respectively, $p = 0.00$; average scores PPL: 13.86 vs. 10.45, respectively, $p = 0.00$; average scores PPB: 10.97 vs. 9.16, respectively, $p = 0.00$).

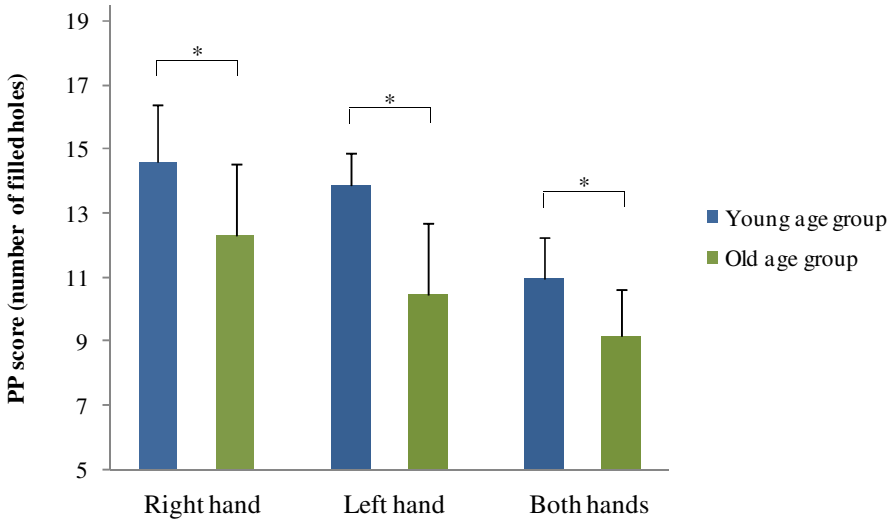

Figure 10. Graph representing the effect of aging on PP hand performance (mean ± SD). The number of filled holes was significantly higher for the young age group compared to the old age group when performing the test with the right hand only, the left hand only and both hands simultaneously. PP, Purdue Pegboard; * $p < 0.05$.

To detect mild cognitive dysfunction in elderly, the old age group completed the MoCA test. All scores were above 20 (mean = 26.33, SD = 1.77), which indicates that none of the test subjects was cognitive impaired.

3.5.1 Behavioral consequences of aging within genotype groups

Next, the effect of aging on executive functioning and fine hand motor performance, taking the two BDNF genotype groups into account, was analyzed. Therefore, the sample was divided in Val/Val and Val/Met carriers. Within the BDNF Val/Val group the Mann-Whitney U-test revealed significant differences between the young age group and the old age group. The younger

Val/Val carriers (*n* = 18) performed significantly better compared to the older Val/Val carriers (*n* = 19) for local/global block 3 ACC (74.56% vs. 62.21% , respectively, p = 0.01; **figure 11**), the PPR hand (average scores: 14.20 vs. 12.10, p = 0.00), PPL hand (average scores: 13.86 vs. 10.33, respectively, p = 0.00) and PPB hands (average scores: 10.76 vs. 9.04, respectively, p = 0.00) **(figure 12)**. For the local/global accuracy scores of block 1+2 however, no significant difference was found (p > 0.05) between old and young Val/Val individuals (data not shown).

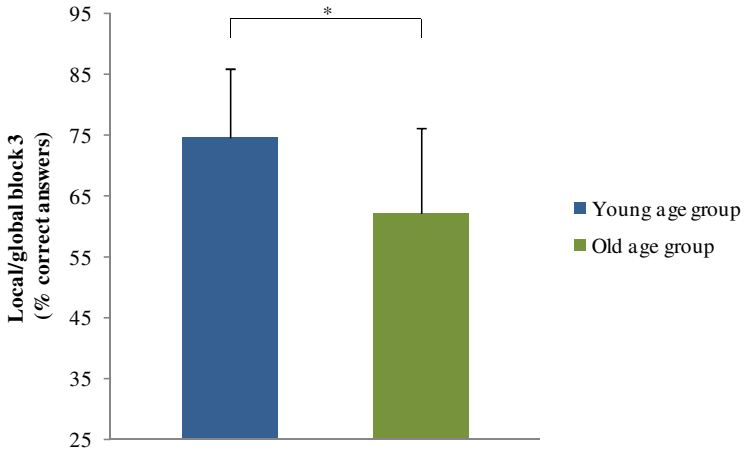

Figure 11. Graph representing the effect of aging on the local/global ACC block 3 scores within the Val/Val BDNF genotype group (mean ± SD). The younger Val/Val carriers performed significantly better compared to the older Val/Val carriers. ACC, accuracy; Val, valine; BDNF, brain-derived neurotrophic factor; * p < 0.05.

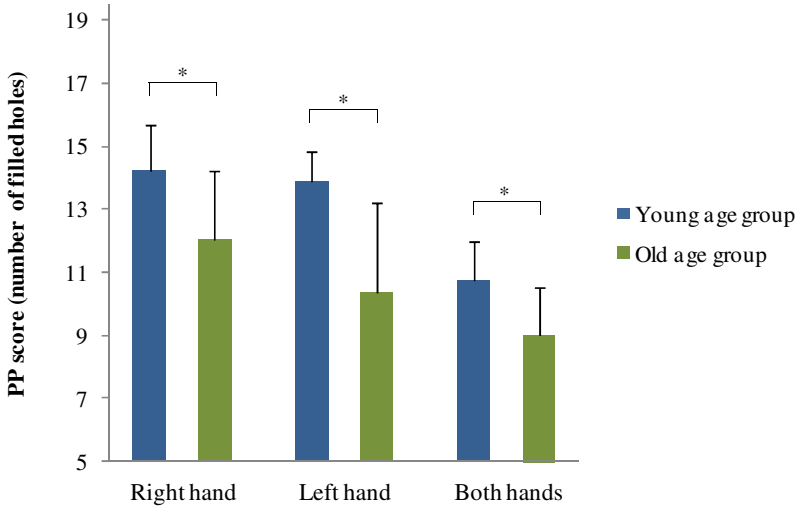

Figure 12. Graph representing the effect of aging on PP hand performance within the Val/Val BDNF genotype group (mean ± SD). The younger Val/Val carriers performed significantly better than the older Val/Val carriers. PP, Purdue Pegboard; Val, valine; BDNF, brain-derived neurotrophic factor; * p < 0.05.

Within the BDNF Val/Met group, the Mann-Whitney U-tests revealed significant differences. The young Val/Met individuals (n = 8) have scored significantly better on the local/global block 3 trials (72.49% vs. 60.51% respectively, p = 0.04; **figure 13**) and have filled significantly more holes compared to the old Val/Met subjects (n = 10) (PPR average scores: 15.63 vs. 12.90, respectively, p = 0.03; PPL average scores: 14.21 vs. 9.37, respectively, p = 0.01; PPB average scores: 11.58 vs. 9.43, respectively, p = 0.01; **figure 14**). For the local/global accuracy scores of block 1+2 however, no significant difference was found (p > 0.05) between old and young Val/Met individuals (data not shown).

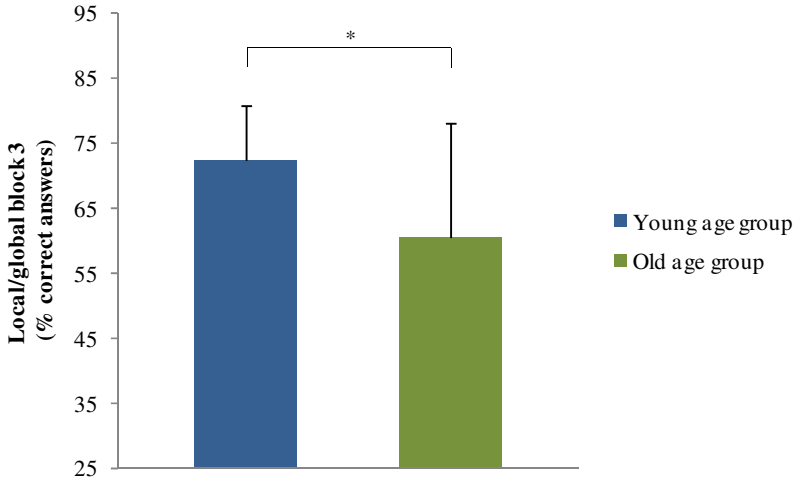

Figure 13. Graph representing the effect of aging on the local/global ACC block 3 scores within the Val/Met BDNF genotype group (mean ± SD). The young Val/Met individuals have scored significantly better on the local/global block 3 trials compared to the old Val/Met subjects. ACC, accuracy; Val, valine; Met, methionine; BDNF, brain-derived neurotrophic factor; * p < 0.05.

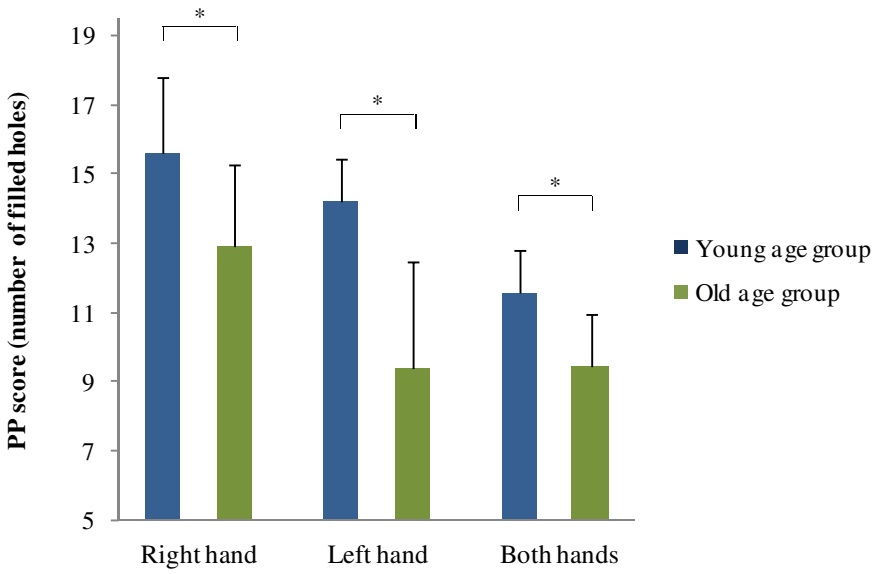

Figure 14. Graph representing the effect of aging on PP hand performance within the Val/Met BDNF genotype group (mean ± SD). The young Val/Met individuals have filled significantly more holes compared to the old Val/Met subjects. PP, Purdue Pegboard; Val, valine; Met, methionine; BDNF, brain-derived neurotrophic factor; * $p < 0.05$.

Subsequently, the effect of aging on executive functioning and fine hand movements, taking the three COMT genotypes into account, was analyzed. However, due to the small sample size of the COMT data (Val/Val young age group, $n = 2$; Val/Val old age group, $n = 1$; Val/Met young age group, $n = 3$; Val/Met old age group, $n = 5$; Met/Met young age group, $n = 3$; Met/Met old age group, $n = 2$) results have to be interpreted with caution. Within the Val/Val and the Met/Met group, for none of the tests a significant difference was found between the young age group and the old age group (all, $p > 0.05$; data not shown). Within the Val/Met group, the Mann-Whitney U-tests revealed a significant difference. The PPL hand score is significantly higher for the young Val/Met individuals compared to the old Val/Met subjects (PPL average scores: vs., respectively, $p = 0.04$, **figure 15**). Other tests showed no significant difference between the young age group and the old age group in the COMT Val/Met group (data not shown).

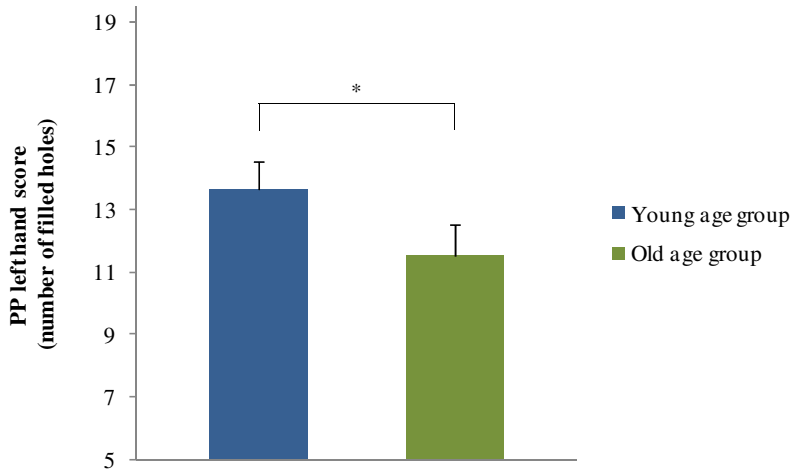

Figure 15. Graph representing the effect of aging on PP left hand performance within the Val/Met COMT genotype group (mean ± SD). The young age group achieved a significantly higher score than the old age group. PP, Purdue Pegboard; Val, valine; Met, methionine; COMT, catechol-O-methyltransferase; * $p < 0.05$.

3.6 Behavioral effects of the BDNF polymorphism

The Mann-Whitney U-tests revealed no statistical significant differences between BDNF Val/Val (n = 37) and Val/Met (n = 18) carriers for the Purdue Pegboard scores and local/global ACC scores (all, $p > 0.05$). The same results were found within the young age group (all, $p > 0.05$) and within the old age group (all, $p > 0.05$; data not shown). To detect mild cognitive dysfunction in elderly, the old age group completed the MoCA test. No significant differences in test outcome were observed between BDNF Val/Val and Val/Met individuals (n = 19 and 10, respectively; $p > 0.05$; data not shown).

3.7 Behavioral effects of the COMT polymorphism

Although the COMT genotype sample size was small, statistical analysis was performed. As a consequence, results were interpreted with caution. No significant performance differences for the Purdue Pegboard scores and local/global ACC scores block 1+2 and block 3 were found between COMT Val/Val (n = 3), Val/Met (n = 8) and Met/Met (n = 5) carriers (all, $p > 0.05$; data not shown). Similar results were found within the young age group (all, $p > 0.05$) and within the

old age group (all, $p > 0.05$; data not shown). Furthermore, no significant difference in the MoCA test outcome was found between COMT Val/Val, Val/Met and Met/Met elderly ($p > 0.05$; data not shown).

3.8 Interaction between BDNF and COMT genotypes in whole subject sample

To detect a gene x gene interaction, the shared contribution of BDNF and COMT genotypes to executive functioning, fine motor hand control, and cognitive ability were analyzed for the whole subject sample. According to the Mann-Whitney U-test, no significant interactional effects of BDNF and COMT genotypes were revealed (all, $p > 0.05$; data not shown). Additional, no trends were observed. However, sample size was small.

4 Discussion

All highly developed nations in the world are experiencing substantial increases in the proportion of elderly due to falling birth rates combined with increased longevity. As a result of this demographic evolution, it is of high importance to understand the behavioral consequences of aging and its determinants. In this senior internship, the aim was to evaluate the influence of aging and genetic variants on human behavior, more specific decision making, fine hand motor control and general cognition skills. Both animal and human studies have suggested that some cognitive deficits observed in aging could be related to disruptions in the dopaminergic and neurotrophic systems[15]. For this reason, genetic polymorphisms that affect the concentration or secretion of neurotransmitters (e.g. DA) and neurotrophic factors (e.g. BDNF) could contribute to some of the individual differences in not only cognitive function, but also motor performance in healthy adults and elderly[22, 58].

4.1 Biomaterial collection and DNA extraction

To maximize the participation rate in population genetic studies, alternatives to invasive whole blood collection are increasing. One such alternative is buccal cell collection, which, in contrast to blood collection is painless, easy to perform by the participant self, fast and inexpensive. Because of these advantages, participants were asked to collect epithelial cells of the internal portion of the cheek by the use of two swabs. Subsequently, DNA was extracted from buccal swabs using the Chelex 100 Resin extraction method. The DNA extraction procedure is simple, rapid (full procedure did not take more than 2 hours) and involves no organic solvents. This makes the protocol suitable for large population genetic studies. Although it is suggested that Chelex DNA extraction method is efficient[83], UV spectrophotometry revealed low quantity and purity in the DNA extract obtained from buccal cell swabs. The low quantity of DNA could be due to several reasons. First, it is possible that components co-purified with the Chelex-extracted DNA had caused DNA degradation during storage. Second, the disruption of cell membranes was possibly insufficient. As a consequence, DNA release from the cells was low. The low DNA purity could be explained by the presence of proteins or other contaminants. To increase quantity and purity, the addition of proteinase K could be helpful. Chai and colleagues (2009) discovered

that this enzyme was essential in protein digestion when using the Chelex DNA extraction method. Furthermore, they revealed that the amount of DNA released from the cells was increased after addition of proteinase K[84]. To achieve optimal DNA amount and purity from buccal cells, different DNA extractions methods and DNA extraction kits should be compared.

4.2 BDNF and COMT genotyping experiments

Previous studies have reported successful PCR amplification after using the Chelex 100 resin DNA extraction method[83, 85]. In our experiment, however, PCR amplification efficiency was low. This could be explained by the fact that success in PCR amplification depends heavily on the quality of DNA template. Lower purity in DNA solution might influence PCR amplification.

4.2.1 Optimization BDNF genotyping protocol

After performing a conventional PCR using the 'standard' DNA *Taq* polymerase, the BDNF sequence of interest was not amplified efficiently. To increase PCR efficiency, a nested PCR was performed. Because nested PCR exists out of two consecutive PCR runs, incorporation of mismatch bases into the extending strand was minimized by the use of a high fidelity PCR kit. The proofreading enzyme Verbatim High Fidelity Polymerase exhibits 3'-5' exonuclease activity that decreases the rate of misincorporation. As a result, the second run of the nested PCR produces a clear 274 bp band (**figure 4**) where the first run was very weak or even invisible. Nested PCR of the BDNF sequence of interest was successful for all participants. Subsequently, 96.5% of the subjects were successfully genotyped for the BDNF gene (55 out of 57 participants). The allelic distributions did not deviate significantly from those expected according to Hardy-Weinberg equilibrium. Note that none of the participants was Met/Met homozygous for the BDNF gene. This is not surprisingly because this genotype represents only 4% of the general population. In conclusion, the BDNF genotyping protocol was successful when using nested PCR to amplify the sequence of interest and DNA sequencing to detect the BDNF polymorphism.

4.2.2 Optimization COMT genotyping protocol

Before starting the amplification of the COMT DNA sequence of interest, two primer pairs were tested (**table 1**). PCR was performed using the 'standard' *Taq* polymerase and 100 ng DNA template extracted from blood cells. Agarose gel showed non-specific DNA and low amplification efficiency of specific DNA after the use of primer pair 1. This could be due non-specific primer binding, duplex and/or hairpin formation of primers. The use of primer pair 2 in amplification reactions, by contrast, resulted in specific and high amounts of PCR product (**figure 6**). Hence, primer pair 2 was chosen to continue amplification of the buccal cell extracted DNA COMT sequence. Unfortunately, PCR products were invisible. Probably, this was again due to the low quality was the DNA template. Another factor that may hinder DNA amplification is the high GC content (62%) of the sequence of interest. Several methods were used to optimize PCR efficiency (**table 2**). First, 5% of DMSO was added to the PCR amplification reactions. This denaturant is known to increase amplification specificity by facilitating DNA strand separation and by destabilizing non-specific primer binding. The addition of DMSO did not resulted in higher PCR efficiency. One should remark that DMSO can inhibit the *Taq* polymerase, which would clarify PCR failure in this case[86]. Second, $MgCl_2$ concentration was enhanced up to 3 mM. Again PCR efficiency was not increased. Finally, two High Fidelity PCR Kits were compared: the HotStar HiFidelity PCR Kit with Q-solution (Q-solution: innovative PCR additive that facilitates amplification of difficult templates by modifying the melting behavior of DNA) and the Verbatim High Fidelity PCR Kit. Although still smearing and primer dimers (**figure 7**) were present, the best results were obtained by using the Verbatim High Fidelity PCR Kit. Factors that may contributed to its success are the use of a 5x GC buffer and the enhancement of the denaturation temperature up to 98°C. Specific 178 bp agarose bands were cut out and DNA was purified. The obtained DNA concentration was sufficient to perform DNA sequencing experiments. Surprisingly, DNA sequencing failed for all samples. To exclude a problem with the sequence primer (forward primer of primer pair 2), the forward primer of primer pair 1 was also tested for sequence PCR. Unexpectedly, when using the forward primer of primer pair 1 sequencing results were adequate: 70.8% of the participants were effectively genotyped for the COMT gene. Due to time problems, only 24 samples were sequenced, of which 17 successfully. All genotypes were consistent with proportions expected under Hardy-Weinberg equilibrium.

A study of Philibert at al. (2008) has found that the use of DNA prepared from whole blood was associated with greater genotyping success[87]. However, since the use of buccal cell-derived DNA has a number of advantages, future investigations may identify methods to optimize DNA purity and quantity, thereby increasing the efficiency of genotyping from buccal cell-derived DNA.
As a conclusion, the COMT genotyping protocol was sufficient when amplifying the DNA sequence of interest at a higher denaturation temperature and by the use of the Verbatim High Fidelity Kit including GC buffer. Then, the resulted PCR product was sequenced to detect the COMT polymorphism. Probably, COMT genotype success rate would increase after additional protocol refinement.

4.3 Behavioral consequences of aging

The first aim of this internship was to investigate the effect of aging on executive functions, fine motor control and cognitive skills. Significant differences were found between the young age group and the old age group. The adults performed significantly better on the local/global task (especially block 3) compared to the elderly. Although the Mann-Whitney U-test revealed also a significant difference between the young and old age group for local/global block 1+2, this seemed improbable. Average scores on block 1+2 were almost equal and standard deviations were small. After calculating frequencies of % correct answers on block 1+2 (**table 4**), it was remarkable that many subjects achieved the same scores. Probably blocks 1 and 2, in contrast to block 3, were not sensitive enough to discriminate between performances of younger and older people. In addition, both age groups scored significantly better on block 1+2 compared to block 3. This is not surprisingly because block 1+2 consist of repeat trials only, which are considered to be 'easy'. In block 3 the large and small cues are being alternated (= repeat trials and switch trials). The combination of repeat trials and switch trials made it harder for the participants to answer fast and accurately. It is well established that normal human aging is associated with decline across a range of cognitive abilities[5]. Neuroanatomical changes, chemical changes and a decrease in white matter integrity all affect the efficacy of neural information transfer, with resulting impairments in the mechanisms supporting executive functioning, memory and global cognition.

Furthermore, adults achieved a significantly higher score on the PP task compared to older persons. Consistent with our results, Smith et al. (1999) have demonstrated a decline in fine motor hand movements in older people[12]. Deterioration in hand function in the elderly population is, to a large degree, secondary to age-related degenerative changes in the musculoskeletal, vascular, and nervous systems. A combination of local structural changes (joints, muscle, bone, nerve and receptors, blood supply and skin) and more distant changes in neural control leads to decreased gross and fine hand motor function in elderly[88].
To discriminate between elderly with mild cognitive impairments and healthy elderly, the old age group completed the MoCA test. All subjects scored above 20, which indicates that none of them was cognitive impaired.

In conclusion, due to the fact that younger subjects achieved higher scores with respect to executive functioning and fine hand motor control, our findings demonstrate that the study subject sample is representative for the general population. Since all elderly scored sufficient on the MoCA and came independently to the test set-up, we have to remark that we studied an active and healthy group of elderly.

4.3.1 Behavioral consequences of aging within genotype groups

Next, the effect of aging on executive functioning and fine motor performance, taking the genotype groups into account, was analyzed. First, the sample was divided in BDNF Val/Val and BDNF Val/Met carriers. In accordance with above mentioned results, the younger subjects of each genotype group performed significantly better compared to the older individuals of each genotype group for local/global block 3 (**figure 11 and 13**), the PPR hand, PPL hand and PPB hands **(figure 12 and 14)**. These results indicate that within each BDNF genotype group, age is the major factor determining performance on the selected tasks. For the local/global accuracy scores of block 1+2 however, no significant difference was found between old and young individuals of each genotype group. This probably results from the low discrimination sensitivity of the task. Second, the sample was divided in COMT Val/Val, COMT Val/Met and COMT Met/Met carriers. Only within the Val/Met group, the Mann-Whitney U-tests revealed a significant difference: the PPL hand score is significantly higher for the young Val/Met individuals compared to the old Val/Met subjects (**figure 15**). However, due to the small sample

size of the COMT data (Val/Val young age group, n = 2; Val/Val old age group, n = 1; Val/Met young age group, n = 3; Val/Met old age group, n = 5; Met/Met young age group, n = 3; Met/Met old age group, n = 2) no conclusions can be drawn.

Important to note is that a great variability exists among aged subjects. A higher standard deviation was observed on performance scores among elderly compared to the young age group. This indicates that cognitive and physical aging is marked by heterogeneity. Longitudinal studies confirm that individual differences in cognitive performance increase from early to late adulthood and suggest that both genetic and environmental factors contribute[89].

4.4 Effects of common polymorphisms on human behavior

The second aim was to examine whether genetic variants, such as the BDNF and COMT genotypes, can influence executive functioning, fine motor performance and general cognitive skills.

4.4.1 Behavioral effects of the BDNF polymorphism

The molecular cascades governing the development and maturation of the CNS are highly conserved in adult organisms and contribute to complex phenomena such as activity-dependent synaptic plasticity. The neurotrophin BDNF is expressed by neurons in response to neuronal activity and it is a critical element in modulating synaptic changes, such as hippocampal LTP, associated with learning and memory. It is known that the BDNF Val/Met polymorphism affects not only the anatomy of the hippocampus, but also has behavioral consequences. Here, we examined whether the BDNF polymorphism affects executive functioning and fine motor hand performance, regardless of age groups and within age groups. The local/global task was administered to assess working memory, inhibition and especially decision making (all properties belonging to executive functioning) in BDNF Val and Met carriers. No significant differences were found between BDNF Val and Met carriers. This result indicates that the BDNF polymorphism does not influence executive functioning, neither in the whole study sample nor within the age groups. In healthy elderly, global cognition was assessed by using the MoCA. All

elderly scored approximately equal and no significant differences were found between Val and Met carriers.

Although some research groups have demonstrated that the BDNF Met allele is associated with short-term memory and general intelligence[44], differences between Val and Met individuals were more pronounced in memory and (motor) learning tasks, that rely heavily on the hippocampus[34, 38, 46]. The local/global task assesses decision making, inhibition and working memory. These processes are rather prefrontal cortex dependent than hippocampal dependent. The lack of a learning factor in our tasks, could probably be an explanation why no differences were found between Val and Met carriers. Another possibility could be that the above mentioned tasks were not challenging enough to discriminate between Val and Met carriers.

To our knowledge, this study was the first investigating the effect of the BDNF genotype on fine hand motor control. No significant difference in PP performance between Val and Met carriers was detected. This finding demonstrates that fine hand motor control was not affected by the BDNF genotype. However, also here, the lack of a learning element in the PP tasks, could explain why no differences were found between Val and Met carriers.

Note that our subject sample solely exist out of healthy adults and healthy elderly. Studies of patient populations suggest that the BDNF polymorphism has been associated with increased susceptibility to pathological conditions such as schizophrenia, Alzheimer's disease, Parkinson's disease, depression and bipolar disorder[90]. A common clinical symptom of these disorders is a varying degree of impairment in cognitive abilities. In a study of Rybakowski et al. (2006), Val/Met individuals who were diagnosed with bipolar disorder performed significantly worse than Val/Val patients on the Wisconsin Card Sorting (WCS) task[91]. The WCS task has classically been used to test executive functioning. This makes several subtests of the WSC task similar to the local/global test we used. As a consequence, it could be possible that the effect of the BDNF Val/Met polymorphism on executive functioning, general cognitive ability and motor control rather would be detectable in pathological conditions than in a healthy subject sample.

In the future, it will be essential to focus on the underlying basis of the BDNF variant's effect on cognitive and motor processes. Several questions remain to be answered. First, it would be interesting to reveal the entire BDNF trafficking machinery. Although one potential trafficking

protein, sortilin, has been identified as being involved in impaired BDNF Met trafficking[92], other components of this trafficking operation are not yet known. Second, information about the extent and nature of the BDNF Met allele's effects on cognitive and motor processes is still missing. Whereas impairments in hippocampal-dependent learning and memory have been shown, it is likely that other cognitive processes will also be affected. Lastly, extensive examination of humans who are homozygous for the BDNF Met allele (represent only 4% of the population) is necessary.

4.4.2 Behavioral effects of the COMT polymorphism

COMT enzymatic activity leads to degradation of DA and thus has an impact on endogenous DA levels in the PFC. The COMT polymorphism is associated with most of the human variation in intrinsic DA levels in the PFC[47]. The resulted Val to Met substitution at amino acid level affects enzymatic activity, which is three to four times higher in Val than in Met homozygotes. Thus, lower enzymatic activity among Met carriers results in less frontal DA degradation and greater DA availability at the receptors[48].

Here, we examined whether the COMT polymorphism affects executive functioning and fine motor hand performance, regardless of age groups and within age groups. No significant performance differences were found between COMT Val/Val, Val/Met and Met/Met carriers for the local/global task, that measures executive functioning. Past research on alleles of the COMT gene and cognition has yielded mixed results. Some studies have demonstrated that in younger adults and in older subjects the Met/Met form of the COMT genotype has been related to superior performance in executive functioning (i.e. fewer errors in the WCS task)[50, 54, 55, 58]. However effect sizes were generally small[50, 51, 55] and not always statistically reliable[93]. Consistent with our study, O'Hara et al. (2006) have found no association between the COMT polymorphism and prefrontal cognitive measures in elderly[61]. Any lack of observation of an association between COMT genotype and the local/global task may be due to the fact that we did not employ measures such as the WCS test or the N-back task. Both tests are widely used to assess executive function and may be more sensitive to deficits in executive functioning than the local/global task employed in the current investigation. However, the most consistent association of the COMT Val allele and poorer prefrontal cognitive function has been observed in patients with schizophrenia, a pathological condition hallmarked by decreases in prefrontal dopamine

activity[94]. Possibly, individuals with normal dopamine activity may be less vulnerable to any negative effects of the Val allele on dopamine signaling. Although it is suggested that older adults experience a loss of dopaminergic function[15], age-related depletions occur across several neurotransmitter systems, and such decrements are likely heterogeneous.
In addition, no significant performance differences between COMT Val/Val, Val/Met and Met/Met carriers for the PP task were revealed. To our knowledge, associations between the Val158Met COMT polymorphism and fine motor hand control in healthy elderly have not been reported yet. Only one study has demonstrated that Met/Val heterozygotes are associated with faster gait velocity as compared to both homozygous forms[72]. Furthermore, no significant difference in the MoCA test outcome was found between COMT Val/Val, Val/Met and Met/Met elderly.

However, a limitation must be considered when interpreting our results with respect to COMT genotype data. Due to circumstances, only 17 subjects were genotyped for the COMT gene. As a result, the sample size was too small to draw conclusions. Furthermore, it is possible that none of the tasks was sensitive enough to detect a behavioral effect of the different COMT genotypes.

4.4.3 Shared contribution of BDNF and COMT genotypes to human behavior

Although gene-gene interactions have been examined in some studies[16, 22], the majority of the studies have been limited to a single SNP. Regardless of age, no significant interactions among the BDNF and COMT genotypes on executive functioning and fine motor hand control were found. Due to the small sample size, it was not feasible to investigate interactional effects within age groups. Our findings are in accordance with the research of Raz et al. (2009)[16]. On the other hand, Nagel and colleagues (2008) did found a COMT x BDNF interaction in elderly[22]. There is a possibility that these reported interactive effects are specific to the cognitive processes they assessed. Cognition refers to the way how humans perceive, remember and learn, cognition is broad and can be assessed in many ways. In this perspective it is possible that gene x or y only contributes to specific cognitive process. Further research into multiple combinations of candidate SNPs and behavioral consequences is needed.

4.5 Limitations of the study

The results of this study should be interpreted in the context of its limitations. First, the sample employed in this study, although more than adequate for examining behavioral effects of aging, might not be sufficiently large for discovering genetic effects. Second, it is a cross-sectional study, in which age-related change can only be inferred, not measured. It is possible that a longitudinal follow-up would reveal more subtle effects of the examined polymorphisms on the selected tasks. Moreover, in a longitudinal study, true change and variability of change can be assessed. Third, the sensitivity of the selected cognitive tasks is questionable. Likely, tasks focusing on very specific cognitive processes have a higher differentiation ability. Finally, humans have more than 30.000 genes and millions of SNPs. It would be unlikely that a specific SNP would play a major role in normal cognitive or fine motor variability.

5 Conclusion

The aim of this senior internship was to investigate the effect of aging and genetic variations on executive functioning, fine motor hand control and cognitive skills. The current study extends previous research associating the polymorphisms with variation in fine hand motor performance. To carry out genotyping experiments biomaterial was acquired through epithelial cell collection of the internal portion of the cheek instead of invasive whole blood collection. As a result, participation rate in this genetic population study was maximized. Genotype success rates were very high for the BDNF gene. COMT genotyping experiments require refinement. Overall, the younger age group performed significantly better on tasks evaluating executive functioning and fine hand movement skills compared to older people. Furthermore, we did not observe any impact of the BDNF or COMT genotype on executive functioning, fine hand motor control and general cognition in healthy adults and healthy elderly. In addition, no COMT x BDNF interaction was revealed. Given the low statistical power of our research, further studies are still necessary to better delineate to what extent genetics contribute to the cognitive and motor functions. As a result of the demographic shift towards an aging society, it is of high importance to understand the underlying mechanisms of behavioral changes in the aging population.

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Supplements

Supplement 1: DNA extraction from buccal cells using Chelex

Required solution

- 5 % Chelex: 0.05g Chelex powder in 1 mL of autoclaved MilliQ

Protocol:

1. Put two swabs with the saliva sample in a 1 mL tube
2. Add 1 mL autoclaved MilliQ to the sample
3. Rotate for 30 minutes at room temperature
4. Centrifuge for 2 minutes at 13000 rpm
5. Discard MilliQ (leave approximately 50 µL at the bottom)
6. Vortex the Chelex solution
7. Add 200 µL of homogeneous suspension of 5% Chelex
8. Vortex
9. Incubate for 30 minutes in a shaker at 56°C
10. Incubate for 8 minutes in a shaker at 95°C
11. Centrifuge for 3 minutes at 13000 rpm
12. Discard swabs
13. Centrifuge for 3 minutes at 13000 rpm (Chelex is now at the bottom)
14. Pipette the supernatants in a new labeled tube (= DNA-sample) and store at -20°C

Supplement 2: Detailed administration and scoring instructions MoCA

1. **Alternerende Trail Making:**

Afname: De onderzoeker instrueert de proefpersoon: "Teken een lijn, van een cijfer naar een letter en in oplopende volgorde. Begin hier [wijs naar (1)] en teken een lijn van 1 naar A, dan naar 2 en zo verder. Stop hier [wijs naar (E)]."

Scoring: 1 punt wordt toegekend indien de proefpersoon het volgende patroon correct tekent: 1-A-2-B-3-C-4-D-5-E, zonder dat de lijnen elkaar kruizen. Een fout die de proefpersoon niet direct zelf verbetert krijgt een score 0.

2. **Visuo-constructieve vaardigheden (Kubus):**

Afname: De onderzoeker wijst naar de **kubus** en geeft de volgende instructie: "Teken dit figuur zo nauwkeurig mogelijk na, in de ruimte hieronder".

Scoring: Er wordt 1 punt toegekend voor een correcte tekening.

- De tekening moet driedimensionaal zijn
- Alle lijnen zijn getekend
- Er is geen extra lijn toegevoegd
- De lijnen lopen relatief parallel en zijn van gelijke lengte (rechthoekige prisma's worden geaccepteerd).

Indien aan één van bovenstaande criteria niet wordt voldaan, is de score 0.

3. **Visuo-constructieve vaardigheden (Klok):**

Afname: Wijs naar de rechter bovenkant van het scoreformulier en geef de volgende instructie: "Teken een **klok**. Plaats er alle cijfers in en zet de wijzers op 10 over 11".

Scoring: Er wordt één punt toegekend voor elk van de volgende 3 criteria:

- Omtrek (1 pt.): de omtrek van de klok moet een cirkel zijn. Hooguit een kleine afwijking is acceptabel (b.v., een kleine onvolkomenheid bij het sluiten van de cirkel);
- Cijfers (1 pt.): alle cijfers van de klok zijn aanwezig, zonder toevoeging van extra cijfers; de cijfers staan in de juiste volgorde en moeten ongeveer in de kwadranten van de klok

geplaatst zijn; Romeinse cijfers zijn toegestaan; de cijfers mogen aan de buitenkant van de cirkel geplaatst worden;

- Wijzers (1 pt.): er moeten twee wijzers zijn die samen de correcte tijd aangeven; de uurwijzer moet duidelijk korter zijn dan de minutenwijzer; de wijzers moeten in de klok getekend worden en elkaar ongeveer in het midden van de cirkel kruizen.

Er wordt geen punt toegekend voor een element indien aan de bovenstaande criteria niet wordt voldaan.

4. Benoemen:

Afname: Wijs vanaf links ieder figuur aan en zeg: "Hoe heet dit dier?".
Scoring: Voor elk van de volgende antwoorden wordt 1 punt gegeven: (1) leeuw, (2) neushoorn, (3) kameel of dromedaris.

5. Geheugen:

Afname: Onderzoeker leest een rij van 5 woorden voor met een snelheid van één woord per seconde, en geeft hierbij de volgende instructies: "Dit is een geheugentest. Ik ga een rij woorden voorlezen die u moet onthouden, nu maar ook straks. Luister goed. Als ik klaar ben, vertelt u me alle woorden die u hebt onthouden. Het maakt niet uit in welke volgorde u ze opnoemt". Zet een kruisje in de aangegeven ruimte voor ieder woord dat de proefpersoon tijdens deze eerste aanbieding reproduceert. Wanneer de proefpersoon aangeeft dat hij/zij klaar is (alle woorden heeft herinnerd), of zich geen woorden meer weet te herinneren, lees dan de lijst met woorden een tweede keer voor met de volgende instructie: "Ik ga dezelfde lijst een tweede keer voorlezen. Probeer zo veel mogelijk woorden te onthouden en vertel ze me, ook de woorden die u de eerste keer hebt opgenoemd." Zet een vinkje in de aangegeven ruimte voor ieder woord dat de proefpersoon zich herinnert na de tweede aanbieding.
Vertel de proefpersoon aan het einde van de tweede aanbieding dat later nogmaals naar de woorden gevraagd zal worden, door te zeggen: "Ik zal u aan het eind van deze test opnieuw vragen welke woorden u zich nog weet te herinneren."
Scoring: Er worden géén punten gegeven voor aanbiedingen één en twee.

6. <u>Aandacht</u>:

<u>Cijferreeksen vooruit: Afname</u>: Geef de volgende instructie: "Ik ga een aantal cijfers opnoemen en als ik klaar ben, moet u ze in dezelfde volgorde nazeggen als ik ze heb gezegd". Lees de vijf-cijfer reeks met een snelheid van één cijfer per seconde.
<u>Cijferreeksen achteruit: Afname</u>: Geef de volgende instructie: "Nu ga ik weer cijfers opnoemen, maar zodra ik klaar ben, moet u ze in omgekeerde volgorde nazeggen." Lees de drie-cijfer reeks met een snelheid van één cijfer per seconde.
<u>Scoring</u>: Er wordt 1 punt gegeven voor elke correct nagezegde reeks, (N.B.: het correcte antwoord voor cijferreeksen achteruit is 2-4-7).
<u>Volgehouden aandacht: Afname</u>: De onderzoeker leest de rij letters voor met een snelheid van één letter per seconde. Geef de volgende instructie: "Ik ga u een reeks letters voorlezen. Iedere keer dat ik de letter A noem, tikt u eenmaal met uw hand op tafel. Wanneer ik een andere letter noem, tikt u niet met uw hand op tafel".
<u>Scoring</u>: Geef 1 punt bij nul of één fout (een fout is een tik bij de verkeerde letter of geen tik bij de letter A).
<u>Seriële 7's: Afname</u>: De onderzoeker geeft de volgende instructie: "Wilt u van 100 zeven aftrekken en van wat overblijft weer zeven aftrekken en zo doorgaan tot ik stop zeg?" Geef deze instructie zonodig tweemaal.
<u>Scoring</u>: Op dit item zijn maximaal 3 punten te behalen. Geef geen (0) punten indien geen enkele correct is, 1 punt voor één correcte aftreksom, 2 punten voor twee of drie correcte aftreksommen, en 3 punten indien vier of vijf aftreksommen juist zijn gemaakt. Tel iedere juiste aftrekking van 7, beginnend bij 100. Iedere aftreksom wordt individueel beoordeeld; dit houdt in dat, indien een proefpersoon met een foutief getal antwoordt, maar vervolgens correct doorgaat met hier 7 van af te trekken, er een punt voor iedere correcte som wordt gegeven. Een proefpersoon kan bijvoorbeeld antwoorden: "92 – 85 – 78 – 71 – 64" waarbij de "92" fout is, maar alle volgende getallen correct zijn afgetrokken. Dit is één fout en het item krijgt een score van 3.

7. <u>Zinnen nazeggen</u>:

<u>Afname</u>: De onderzoeker geeft de volgende instructies: "Ik ga u een zin voorlezen. Zeg deze na zodra ik klaar ben, precies zoals ik hem heb gezegd [pauze]: **Ik weet alleen dat Jan vandaag geholpen zou worden."** Na het antwoord zegt u: "Nu ga ik u een andere zin voorlezen. Zeg deze na, precies zoals ik

hem heb gezegd [pauze]: **De kat verstopte zich altijd onder de bank als er honden in de kamer waren.**"

Scoring: Ken 1 punt toe voor iedere correct herhaalde zin. De herhaling moet precies hetzelfde zijn. Wees alert voor omissies (b.v., "alleen", "altijd" vergeten) en vervangingen/toevoegingen (b.v., "Jan is degene die vandaag heeft geholpen"; "verstopte" vervangen door "verstopt", meervoud veranderen, etc.).

8. Verbale fluency:

Afname: De onderzoeker geeft de volgende instructie: "Noem zo veel mogelijk woorden als u kunt bedenken die beginnen met een bepaalde letter van het alfabet. Ik zal u de letters straks vertellen. U mag ieder woord noemen dat u wilt, behalve namen, cijfers, of woorden die met hetzelfde voorstukje (voorvoegsel) beginnen, zoals bijvoorbeeld lief, liefde, liefdevol. Na één minuut vraag ik u te stoppen. Bent u er klaar voor? [pauze] Noem zo veel mogelijk woorden als u kunt bedenken die beginnen met de letter **D**. [tel 60 sec af]. Stop."

Scoring: Ken 1 punt toe indien de proefpersoon 11 woorden of meer kan opnoemen in 60 seconden. Noteer de antwoorden onderaan het blad, of in de kantlijn.

9. Abstractie:

Afname: De onderzoeker vraagt de proefpersoon uit te leggen wat ieder woordpaar gemeenschappelijk heeft. Begin met het voorbeeld: "Kunt u mij vertellen in welke opzicht een sinaasappel en een banaan aan elkaar gelijk zijn, wat is de overeenkomst tussen beide?". Wanneer de proefpersoon een concreet antwoord geeft, zeg dan slechts één keer extra: "Weet u nog een andere overeenkomst?". Indien de proefpersoon niet het correcte antwoord geeft (fruit), zeg dan, "Ja, en het is beide fruit." Geef geen extra instructies of verduidelijking.

Na de oefenafname, zegt u: "In welk opzicht zijn een trein en een fiets aan elkaar gelijk?". Nadat het antwoord gegevens is, stelt u een tweede vraag: "Vertel me nu in welk opzicht een liniaal en een horloge aan elkaar gelijk zijn". Geef geen extra instructies of aanmoedigingen.

Scoring: Alleen de laatste twee itemparen worden gescoord. Geef 1 punt voor ieder correct beantwoord itempaar. Deze antwoorden worden goedgekeurd:

Trein-fiets = vervoermiddelen, manieren om te reizen, je kunt met beide tochten maken;

Liniaal-horloge = meetinstrumenten, worden gebruikt om te meten.

De volgende antwoorden worden **niet** goedgekeurd: Trein-fiets = zij hebben wielen; Liniaal-horloge = zij hebben cijfers.

10. Uitgestelde recall:

Afname: Onderzoeker geeft de volgende instructie: "Ik heb u eerder een rij met woorden voorgelezen, en ik vroeg u ze te onthouden. Vertel me zo veel mogelijk woorden die u zich kunt herinneren. Zet een vinkje in de daarvoor bestemde ruimte (√) voor ieder correct woord dat de proefpersoon zich spontaan, zonder aanwijzingen, heeft weten te herinneren.
Scoring: Ken 1 punt toe voor ieder woord dat spontaan wordt herinnerd zonder aanwijzingen.

11. Oriëntatie:

Afname: Onderzoeker geeft de volgende instructie: "Vertel me de datum van vandaag". Indien de proefpersoon een onvolledig antwoord geeft, moedig hem dan aan door te zeggen: "Vertel me het [jaar, maand, precieze datum, en dag van de week]." Zeg vervolgens: "Vertel nu: hoe heet dit gebouw en in welke stad/plaats zijn we nu?"
Scoring: Geef 1 punt voor ieder correct beantwoord item. De proefpersoon moet de exacte datum en het exacte gebouw noemen (naam van het ziekenhuis, kliniek, kantoor). Er worden geen punten toegekend als de proefpersoon er één dag naast zit wat betreft de dag van de week en de datum (dag van de maand).

TOTALE SCORE: Tel alle subtestscores bij elkaar op. Tel er 1 punt bij op voor personen die 12 jaar of minder formele opleiding hebben gehad (gerekend vanaf leeftijd 6 jaar), zodat een maximum van 30 punten mogelijk is. Een uiteindelijke score van 20 of hoger wordt beschouwd als normaal.

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Richting: **master in de biomedische wetenschappen-klinische moleculaire wetenschappen**
Jaar: **2011**

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Voor akkoord,

Bogaers, Lise

Datum: **14/06/2011**