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Genetics of Brain Aging:
Heritability of Neuropsychological Abilities

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Kurzfassung

Das zunehmende Risiko an neurodegnerativen Demenzformen zu erkranken und dem folglichen Verlust eines unabhängigen Lebens wirft die Frage auf wie kognitiv erfolgreiches Altern funktioniert. Es wird angenommen, dass Gene in verschiedenen Ebenen, wie die physiologischen Effekte von Nervenzellaufbau, Myelinisierung und dendritisches Wachstum, die kognitive Phänotypgenerierung beeinflussen. In dieser Studie wurde univariate Heritabilität in verwandten und unverwandten Individuen für kognitive Eigenschaften mit familienbasierendem Ansatz und einem Ansatz mit Genotypdaten berechnet. Zusätzlich wurde die bivariate Korrelation von wahrscheinlich vererblichen kognitiven Eigenschaften untersucht. Die Domänen Gedächtnis, exekutive Funktionen und motorisch Fähigkeiten zeigten moderate bis hohe Heritabilität in der untersuchten verwandten Population, die Berechnung mittels häufigen genetischen Variationen wiesen etwa die Hälfte der Heritabiliät auf. Die Domänen Gedächtnis und exekutive Funktion korrelierten stark, dies lässt gemeinsame genetische Hintergründe vermuten. Eine moderate Korrelation der Umwelteinflüsse war zwischen exekutiver Funktion und motorische Fähigkeit zu beobachten. Durch die Berechnung der Heritabilität mittels Genotypdaten ist es möglich in einem angemessenen Probenumfang teilweise den Anteil der genetischen Faktoren der Phänotypvarianz aufzudecken. Seltene genetische Varianten sind Teil der fehlenden Heritabilität und tragen womöglich zu der kognitiven Phänotypvarianz bei. Die bivariaten Ergebnisse lassen daraus schließen, dass es Gene gibt die mehrere Phänotypen beeinflussen. Weitere Studien sind notwendig um genetische Faktoren, aber auch Umweltfaktoren zu identifizieren die kognitive Eigenschaften beeinflussen um in weiterer Folge mögliche Faktoren für Demenzerkrankungen zu finden.

Abstract

In times of awareness of the increasing risk of neurodegenerative diseases like dementia and the entailing loss of independent living, the question of successful cognitive aging is getting more and more foregrounded. It is assumed that genes influence several stages of cognitive phenotype generation, like the physiological effect of neural plasticity or physical effects like myelinization and dendritic complexity. Univariate heritability in related and unrelated samples was estimated for cognitive traits using family- and genotyping-based approaches. Furthermore we investigated bivariate correlation for probable heritable cognitive abilities. Three of four domains, memory, executive function and fine motor skills, revealed a moderate to high heritability in the family study, estimates computed with common SNPs accounted for about half of the family-based results. The memory domain showed strong correlation to executive function indicating for shared genetic factors, moderate environmental correlation was observed for executive function and fine motor skills. Approaches using common SNPs are able to detect genetic factors associated with cognitive abilities in an adequate large sample size partially. Rare variants are part of the missing heritability and might include variants contributing to the phenotypic variance of cognitive abilities. The bivariate approach give rise to detect generalist genes included in the pathway from genes to brain function to cognitive performance. Further studies are needed to find genetic and environmental factors contributing to cognitive abilities to further identify causal genes in the pathology of disorders.

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1 Introduction

Brain aging and cognitive decline

In times of awareness of the increasing risk of neurodegenerative diseases like dementia and cognitive impairment and the entailing loss of independent living, the question how to avoid this in late-life is getting more and more foregrounded in public life. It is widely believed that the normal aging process including behavioral alterations and moderate cognitive decline is not a single factor observation, but more a multifactorial process. Reviewed studies on non-demented human individuals and animal studies showed that brain morphological changes occur during the process of aging [1]. Observation of regional loss of neurons are made, but not in an extent amount. Decreased branching and shortening of dendrites as well as axonal degeneration enabled by possible accumulation of filaments, modified mitochondria or glycogen inclusion are found in aging brains. Myelin sheath structures are affected too and are expected to reduce the conduction velocity that interrupts the neuronal synchronicity. In a study of aged rhesus monkeys a loss of synapses was observed [2]. Those age-related processes influence the regeneration by delaying the mechanism as well as the information processing through missing inputs at synapses and altered conductive velocity [1]. In aged individuals a reduction of the capacity for information processing, needed for handling of cognitive challenges, leads to reduced cognitive performance [3]. A probable process counteracting cognitive decline is called cognitive reserve [3, 4]. It describes the capability to response to tasks in alternate cognitive approaches or even another neural network until a certain threshold of morphological changes is exceeded. Volume changes in specific brain regions during aging are supposed to affect cognitive abilities. Several reviewed studies found an association between the shrinkage of regional brain volumes and cognition [5]. For example, association of the enthorinal cortex and weaker memory performance in a small non-demented sample size was found [6]. Normal brain aging includes cognitive decline as well, but in obvious inferior form as in demented individuals.

Dementia and its subtypes

The prevalence of developing dementia was estimated to be 7 % for in Western Europe at an age older than 60 years and was found to be higher for females [7]. Eighteen percent of the Austrian population is older than 65 years published in the population record of STATISTIK AUSTRIA in 2013, according to this more than 100000 people suffer from dementia in Austria [8]. Several types of dementia and mixed forms occur. The four most common types are Alzheimer's Disease, Vascular Dementia, Dementia with Lewy Bodies and Frontotemporal Dementia. Alzheimer's Disease is responsible for approximately 70 % of all dementia cases is a progressive neurodegenerative form of dementia [9]. Vascular Dementia is the second most common form and show degeneration of neurons due to impaired cerebral circulation [10]. Dementia with cytoplasmic α-synuclein inclusions, so called Lewy Bodies, in neurons displays in addition to dementia mild parkinsonian syndrome. The type of Frontotemporal Dementia shows progressive brain atrophy especially in frontal lobes [11]. Decreasing neuropsychological abilities are observed in individuals suffering from dementia [12]. Memory, orientation, reasoning as well as cognition in general are negatively affected. Finally alteration of personality and neurological failures appear.

Genetics of brain aging and dementia

Changes in neuropsychological phenotypes in older age are influenced due to structural alterations, biochemical and genetic contributions. Two recent and substantial reviews examined the genetic and environmental elements influencing the aging process and cognitive decline [13, 14]. Aging of the brain is influenced by genetic and environmental factors, whether promoting or acting against the mechanisms of aging. Studies on the genetics of Alzheimer's Dementia (AD), responsible for most dementia cases, revealed susceptible genes for late-onset of AD [15, 16]. One example is the apolipoprotein E gene *(APOE*). It was confirmed to influence the risk of AD in a dose-dependent manner [17, 18, 19]. Three disease-linked polymorphisms were detected, the strongly increased risk associated with the *APOE* ε4 allele, the decreased risk associated with the *APOE* ε2 allele and the neutral risk with the *APOE* ε3 allele [20].

Few studies have been published on the genetics of successful cognitive aging, where intact cognitive abilities retain up until the old age [16]. It is assumed that genes influence several stages of the phenotype generation, like the physiological effect of neural plasticity or physical effects like myelinization and dendritic complexity [21]. It is therefore to expect that cognitive phenotypes are multifactorial with both genes and environment playing an important role and that many genes will have pleiotropic effects and will influence multiple cognitive domains and eventually global cognitive ability.

Longevity and cognitive functioning as a whole might be genetically too complex to find particular genetic factors. No genes of large effective size have been identified so far, leading to the suggestion that cognitive abilities are complex traits with heritability composed of genes contributing to the trait with small effective size [22, 23]. Heritability estimation of complex traits is beneficial to get a general view if genetic effects are relevant for the variance of a phenotype.

Furthermore environmental factors like physical and mental inactivity are adversely influencing neuronal degeneration [24]. The education level is supposed to play a major role in cognitive performance and might be part of the cognitive reserve. A study on this environmental factor revealed interesting results in woman ranging in age between 70 and 79 [25]. Education predicted cognitive performance and decline in woman with higher education in younger years. Another study investigating white matter lesion and the risk of developing dementia in low and high educated healthy subjects revealed association of higher education levels and the lower risk of dementia in individuals with white matter lesions [26]. Environmental factor more or less influence certain cognitive abilities and therefore may not be excluded in heritability studies.

Heritability

The proportion of the total cognitive ability variance in a population that is due to genetic differences between individuals is termed as heritability. High heritability estimates are primarily caused by genotypic differences leading to a phenotypic variation, rather than environmental conditions or stochastic effects. In contrast, low heritability indicates that the trait variability is predominantly environmentally or stochastically influenced and the genotypic variability contributes in a minor role to the trait variability [23, 27].

The limitations of heritability are that the estimation is time- and population-specific, as well as sensitive to environmental conditions. For example, if the environmental variance decreases due to homogenous circumstances and the genetic variance remains constant, then the heritability will increase [27, 28, 29].

The indication of the main factor contributing to the phenotypic variance is the basic outcome of estimating a trait's heritability. Either trait variability is influenced by genetic differences or by environmental conditions and random effects [27, 28, 29].

Assuming that a phenotype (P) is the determined through genetic (G) and environmental effects (E), it is modeled as follows:

$$P = G + E$$

Genetic factors are categorized according to additive genetic variance meaning that contribution of multiple genes are added resulting in the phenotypic response, and to the fact that interactions among genes occur and influence the phenotype [27, 28, 29].

The genetic effects are grouped into three categories. First the additive genetic effects (A), meaning that the effect of adding up each genotypic locus resulting in the phenotypic response. The second and third categories are termed as non-additive effects, here genetic dominance effects (D) and epistasis (I) are distinguished. The former include interactions within a gene locus, where the maternal and paternal allele at a given locus interact. The latter category comprises effects due to interactions between various genes influencing the phenotypic variance [27, 28, 29].

Environmental effects are distinguished into shared (C) and non-shared (E) environmental conditions. The former ones are contributing to similarities and the latter ones to dissimilarities between individuals [27, 28, 29].

The formula from above is extended with the subcategories of genetic and environmental effects to:

$$P = A + D + I + C + E$$

The variance of a trait (V_P) behaves equally as for a phenotype, where the variables VA, V_D, V_I and V_C and VE to genetic (V_G) and environmental effects (V_E), respectively:

$$V_P = V_G + V_E$$

or:

$$V_P = V_A + V_D + V_I + V_C + V_E$$

Heritability is defined as the ratio of genetic variance to phenotypic variance. Two types of heritability are distinguished, broad sense heritability (H^2) and narrow sense heritability (h^2). The first one considers both categories of genetic factors, whereas estimation of the narrow sense heritability includes only the additive genetic factors [27, 28, 29].

H^2 considers all three categories of genetic effects, additive and non-additive effects, given as:

$$H^2 = \frac{V_G}{V_P}$$

or:

$$H^2 = \frac{(V_A + V_D + V_I)}{V_P}$$

In contrast estimation of the narrow sense heritability includes only the additive genetic effects:

$$h^2 = \frac{V_A}{V_P}$$

Heritability estimates of a family-based study with mean age of 73.3 years on cognitive functions revealed moderate to low estimates, 42 %, 53 %, 35 % and 9% for the neuropsychological summary, memory domain, executive functions and attention, respectively [16]. A twin-based study with individuals at age 12 were investigated regarding their heritability estimates of neuropsychological phenotypes and the general cognitive ability based on genotype data as well as twin-based estimation [30]. Estimates for the general cognitive ability of 35 % based on genotype and 46 % based on twin-based data were found. Heritability estimates derived from family-based approaches seems to be higher, those might be biased by shared environmental factors as well as non-additive effects. Whereas estimates based on genotype data only comprises additive genetic effects.

Here we present the evaluation of univariate heritability estimates examined on cognitive ability phenotypes in a non-demented population-based study as well as in a family-based study. The purpose of this study is to compare the heritability using genotyped common SNPs for the estimation in the non-related population and the estimation using family structure information including additive and non-additive genetic effects and shared environmental factors. Furthermore we investigated genetic and environmental correlation for probable heritable cognitive abilities.

2 Materials and Methods

Study subjects

The subjects were from the Austrian Stroke Prevention Study (ASPS), including randomly selected individuals from the city of Graz aged 44 to 75 years. The single-center prospective follow-up study having 2008 samples has been approved by the Medical Ethics Committee of the Karl-Franzens University of Graz.

The Austrian Stroke Prevention Family Study (ASPFS) comprises 182 families including 418 study subjects. The study is composed of the index study subjects of the ASPS and their family members reflecting a population based study of Graz. The study is approved by the Medical Ethics Committee of the Medical University of Graz.

Phenotypic overview

The specific domains of the overall neuropsychological summary and the implemented tests were:

Memory domain

Bäumler's Lern- und Gedächtnistest is composed of six subtests for verbal and figural memory. The verbal memory is tested by repeating telephone numbers, Turkish vocabularies and details of a construction report. The figural memory is examined through memorizing a city map, various items and company logos.

Executive function

Three tests were administered for the executive function domain. In Trail Making Test B the examinee is asked to connect numbers and letters in consecutive alternating order. The second test is called Digit Span Backward a task of the Wechsler Adult Intelligence

Scale IV, here a sequence of numbers is read and then recalled in reverse order by the subject. During the Wisconsin Card Sorting Test four cards are shown, a fifth card has to be assigned either by color, shape or number by the subject in several repetitions.

Attention and Speed

For the Alterskonzentrationstest a figure is given and has to be crossed out among a similar set of figures. In the ASPS the Wiener Reaktionsgerät after Schuhfried, where the test person is asked to press a certain button when a particular acoustic or color stimulus is given, was used for this domain additionally.

Fine motor skills

For the fourth domain the Perdue Pegboard Test (Tiffin 1986) was applied. Here, metal pins are placed into a row of holes as fast as possible by either one or both hands at a time.

The organization of the examined phenotypes is shown **Figure 1**. Some subtests were reverse-scored as required and afterwards z-score transformation was applied. Each cognitive domain was estimated by the sum of standardized scores of sets of one or more neuropsychological measures divided by the number of included measures. The overall neuropsychological summary score was estimated by the sum of z-score of all domains divided by the number of domains. Outliers above or below two standard deviations from the mean were excluded for each cognitive domain and neuropsychological summary score. Almost normal distributed measures were present for analysis.

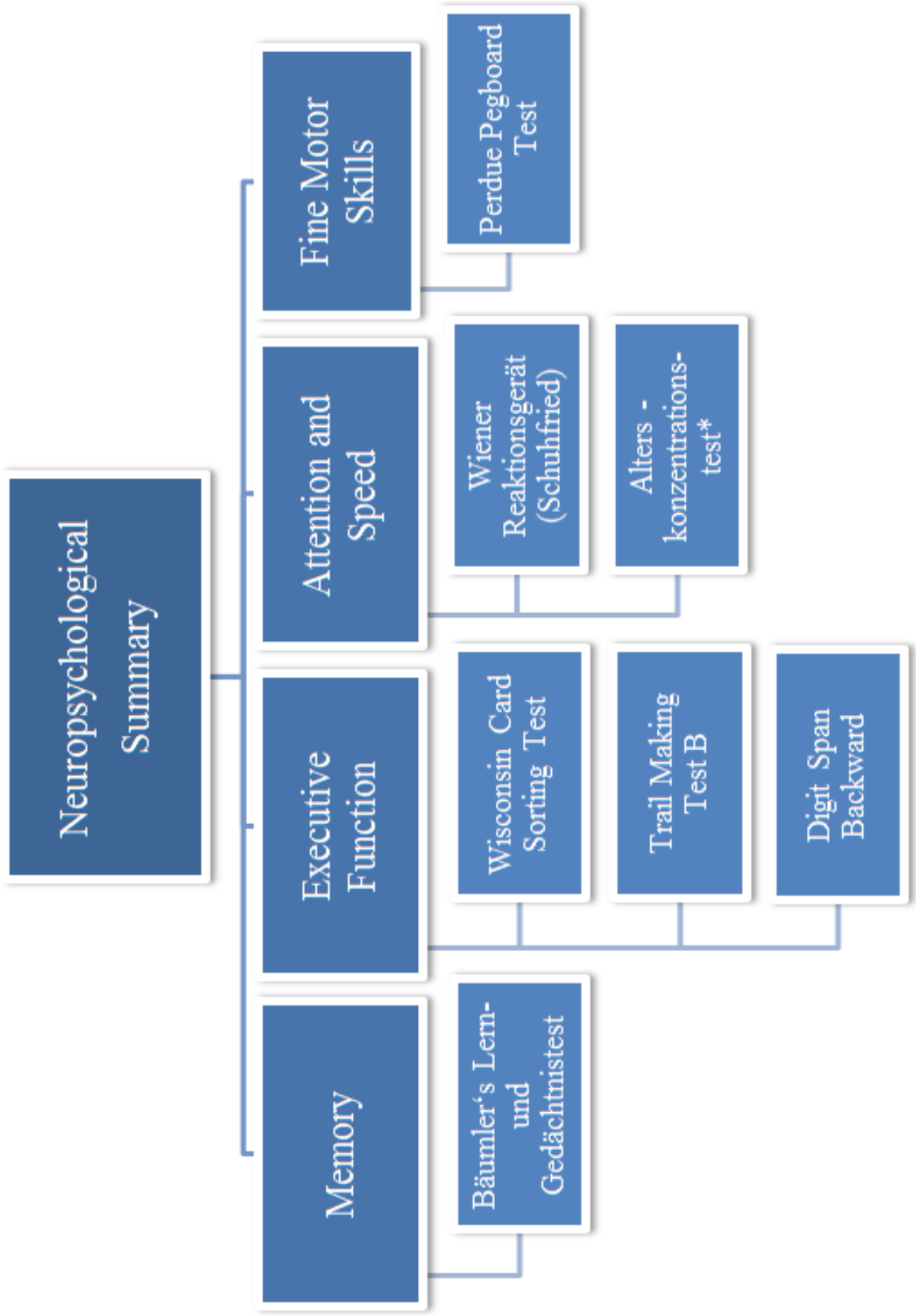

Figure 1. Domains of neuropsychological phenotypes and corresponding tests. *Alters-konzentrationstest was additionally used for the attention and speed domain in ASPS only.

An overview of demographic characteristics is shown in **Table 1** and of phenotypic characteristic in **Table 2**.

Characteristic	ASPS	ASPFS
Demographics		
n	479	376
mean age [years] (SD)	63.63 (7.38)	64.58 (10.78)
female n (%)	263 (54.9)	226 (60.1)
mean education [years] (SD)	11.32 (2.70)	11.74 (3.12)
APOE genotypes		
2|2 n (%)	5 (1.0)	4 (1.1)
2|3 n (%)	58 (12.1)	40 (10.6)
2|4 n (%)	6 (1.3)	2 (0.5)
3|3 n (%)	326 (68.1)	269 (71.5)
3|4 n (%)	82 (17.1)	57 (15.2)
4|4 n (%)	2 (0.4)	4 (1.1)

Table 1. The demographic characteristics of ASPS and ASPFS.

Characteristic	ASPS	ASPFS
Cognitive phenotypes	Mean (SD)	Mean (SD)
Neuropsychological Summary*	0.01 (0.43)	0.00 (0.63)
Memory*	-0.07 (0.80)	0.00 (1.00)
Lern- und Gedächtnistest [a)]	38.36 (10.89)	45.01 (16.06)
Executive Function*[b)]	0.08 (0.46)	0.00 (0.70)
WCST: Perseverative Response	95.60 (6.06)	95.73 (8.71)
WCST: Errors	68.94 (11.70)	65.19 (12.93)
Trail Making Test B	184.70 (49.07)	223.76 (56.42)
Digit Span Backward	4.70 (1.27)	4.21 (1.27)
Attention and Speed*	0.04 (0.35)	0.00 (0.60)
AKT: Time	83.96 (11.88)	na (na)
AKT: Errors	12.82 (0.50)	na (na)
AKT: Correct	19.35 (1.05)	na (na)
WR: Reaction Time	310.68 (116.57)	292.15 (118.42)
WR: Errors: false reacted	10.68 (0.75)	6.85 (0.59)
WR: Errors: not reacted	5.67 (0.60)	9.69 (0.74)
WR: Errors: partial reacted	6.46 (0.79)	7.33 (1.09)
Fine Motor Skills*	0.00 (0.81)	0.00 (0.92)
Right/Left/Two-handed	38.08 (5.23)	36.13 (5.98)
Assembly	27.17 (5.90)	24.80 (6.86)

Table 2. The characteristics of cognitive phenotype and their included subtests of ASPS and ASPFS. *Mean of cognitive domains are Z scores. WCST – Wisconsin Card Sorting Test. AKT – Alterskonzentrationstest. WR – Wiener Reaktionsgerät. [a)] Sum score of the six Lern- und Gedächtnistest subtestes. [b)] Additionaly the Achieved Categories of the WCST were used for Executive Function score.

Genotyping

DNA extraction

Genomic DNA was isolated from whole-blood samples using phenol-chloroform extraction. Cell membranes of leukocytes were broken using TKM1 in a volume ratio of 1:1 to the sample volume. Nuclei were pelletized by centrifugation at 2200 rpm and 20 °C for 10 min. The supernatant was discarded and the pellet was resuspended in TKM I using the same volume as before and again centrifuged. To prevent interfering reactions during PCR caused by remaining iron, the former step was repeated until clearness was achieved.

The pellet was resuspended in 160 µl TKM2 per ml of used whole-blood. 20 µl of proteinase K (10 mg/ml) was added for protein digestion to remove contamination through remaining proteins and to prevent DNA destruction through DNases. In addition 20 µl SDS (10 %) was added to denature proteins and thus activate proteinase K. 800 ml TKM2, 100 µl proteinase K (10 mg/ml) and 100 µl SDS (10 %) was used for whole-blood samples exceeding a volume of 5 ml. The mixture was incubated at 950 rpm and 48 °C for 16 to 22 hours.

After incubation 500 µl phenol-chloroform-isoamyl alcohol in a ratio of 25:24:1, respectively, was added, vortexed and centrifuged at 16100 g and 20 °C for 5 min. The upper aqueous phase containing nucleic acids was transferred into a new tube. 500 µl of chloroform was added, vortexed and centrifuged as before. The upper phase was transferred into a 15 ml tube. DNA was precipitated by adding ethanol (100 %) in a sixfold volume and gently swaying. For purification the precipitated DNA was transferred to a new tube, then washed and centrifuged with 500 µl ethanol (70 %) at 16100 g and 20 °C for 1 min. The washing step was repeated twice. Afterwards 500 µl ethanol (100 %) was added, the mixture was swayed and centrifuged as before. The supernatant was discarded and the pellet was dried at room temperature for 30 min. The pellet was resuspended with 100 µl TE buffer (10 mM, pH 8,8) and solved at 4 °C overnight.

The DNA yield was measured by photometric measurement at DNA absorbance maximum at 260 nm. Protein absorbance maximum at 280 nm was measured due to contamination validation. DNA with A_{260}/A_{280} ratio between 1,7 and 2,0 was denoted as good-quality DNA. Additionally purified DNA was loaded for degradation on 1,5 % 1xTBE agarosegel containing EtBr (1 %) in 1xTBE buffer at 100 V for 25 min and checked under UV-light.

Only non-degraded good-quality DNA was stored at 4 °C as working DNA used for further analysis adjusted to 100 ng/µl and concentrated aliquots at -70 °C.

Genotyping using Affymetrix Genome-Wide Human SNP Array 6.0

DNA samples were genotyped on the Affymetrix Genome-Wide Human SNP Array 6.0 according to the manufacturer's protocol.

In brief 250 ng DNA of each sample was digested using the restriction enzymes NspI (10 U/µl) and StyI (10 U/µl) separately. Adaptors carrying the sequence of complementary NspI or StyI restriction sites were ligated using T4 DNA Ligase (400 U/µl) (New England Biolabs, Ipswich, MA, USA) to the DNA fragments.

Amplification of DNA fragments was done using the universal PCR Primer 002 (100 µM) that recognizes the adaptor's primer site and the Titanium DNA Amplification Kit (Clontech, Mountain View, CA, USA) containing dNTPs (2,5 mM), GC-Melt (5 mM), TITANIUM™ Taq DNA Polymerase (50x) and TITANIUM™ Taq PCR Buffer (10x). The initial denaturation was done at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 60 °C for 45 sec and elongation at 68 °C for 15 sec and finally an extension at 68 °C for 7 min was performed. The preferable fragment size range of 200 to 1100 bp was checked after sample application on a 1 % TAE (1x) gel stained with ethidium bromide at 100 V for 50 min. The gel was exposed to UV-radiation and pictured.

The amplified fragments were purified according to the isopropanol purification method. Briefly, pooled PCR products were incubated with EDTA (0,5 M), then the DNA fragments were precipitated by adding a mix of ammoniumacetate (7,5 M) and isopropanol (99 %). After centrifugation the pellets were washed with 75 % ethanol and dissolved with EB buffer (10 mM Tris-HCl, pH 8,5). The optical density of each PCR product was measured at the absorbance maximum of double-stranded DNA with a wavelength of 260 nm. The PCR product concentration range, preferable 450 to 600 ng/µl, was determined. Furthermore the PCR products were measured to detect contamination at the protein absorbance maximum of 280 nm and at particulates absorbance maximum of 320 nm.

The fragmentation of the purified samples using DNaseI (0,1 U/µl) was proceeded at 37 °C for 35 min and stopped at 95 °C for 15 min. The fragmentation products were checked for the preferable fragment size of less than 200 bp on a 3 % TAE (1x) gel stained with ethidium bromide at 100 V for 40 min. The gel was exposedd to UV-radiation and pictured.

Afterwards the fragmented amplicons were end-labeled using a biotin-labeling reagent (30 mM) and the enzyme terminal deoxynucleotidyl transferase (30 U/µl). The labeled fragments were added to a mixture containing MES (1,25 M), Denhardt's Solution (50x), EDTA (0,5 M), Hering Sperm DNA (10 mg/mL), Oligo Control Reagent 0100, Human Cot-1 DNA (1 mg/mL), 3 % Tween-20, 100 % DMSO and TMACL (5 M). The hybridization mix was denatured at 95 °C for 10 min, cooled to 49 °C and immediately transferred on the Genome-Wide Human SNP Array 6.0. After hybridization the hybridization mix was replaced by an array holding buffer containing MES (100 mM), NaCl (1 M) and 0,01 % Tween-20.

The arrays were stained according to the GenomeWideSNP6_450 protocol for the Fluidics Station 450 (Affymetrix, Santa Clara, CA, USA). Mixtures of SAPE (Molecular Probes, Life Technologies, Carlsbad, CA, USA) (10 µg/mL), SSPE (6x), 0,01 % Tween-20 and Denhardt's Solution (1x) and biotinylated antibody (Vector Laboratories, Burlingame, CA,

USA) (5 µg/mL), SSPE (6x), 0,01 % Tween-20 and Denhardt's Solution (1x) were used for staining. The washing steps were performed using a non-stringent wash buffer containing SSPE (6x) and 0,01 % Tween-20 and a stringent wash buffer containing SSPE (0.6x) and 0,01 % Tween-20. The arrays were scanned by the GeneChip Scanner 3000 7G (Affymetrix, Santa Clara, CA, USA).

For quality control the GeneChip Command Console AGCC Software (Affymetrix, Santa Clara, CA, USA) was used. As quality control all samples were verified for intensity contrast and call rate for a set of probes. Low-quality samples were excluded if the assay criteria were not achieved. Additionally the computed gender was compared to existing data.

SNP Genotyping

The calling of the genotypes for each individual was done by Edith Hofer (Department of Neurology of the University and State Hospital of Graz) and Paul Freudenberger (Department for Molecular Biology and Biochemistry of the Medical University of Graz). The Genotyping Console v4.1 (Affymetrix, Santa Clara, CA, USA) was utilized for genotyping using the Birdseed genotype calling algorithm. In brief, this algorithm builds models from HapMap data for every SNP on the array at first. In phase two genotyping is done by dedicating each SNP to the according model by an expectation maximization algorithm. Birdseed produces genotypes and confidence scores for each SNP of each individual.

A filtering step was conducted to the genotyping. The included criteria are composed of minor allele frequency, Hardy-Weinberg equilibrium p-value, sample call rate and SNP call rate. Alleles with a minor allele frequency of less than 5 % were excluded. Genotypes with Hardy-Weinberg equilibrium p-value less than $5x10^{-3}$ were omitted. Samples with an overall sample call rate less than 98 % and a SNP call rate less than 98 % were filtered out.

Heritability estimation

The univariate heritability estimation was done using two different software tools, the Sequential Oligogenic Linkage Analysis Routines (SOLAR) software [31] and the Genome-wide Complex Trait Analysis (GCTA) tool [32].

Heritability estimation using SOLAR

The SOLAR v.6.6.2 package was used to perform a variance components analysis of family based data in the ASPFS. Maximum-likelihood estimation is applied to a mixed effects model, the total variance of a trait is decomposed into additive genetic, fixed covariate and residual effects. It is assumed that each component has an effect on the trait. The pedigree structure of the subjects was prepared. The covariates likely to influence the examined phenotypes, gender, age, year of education and *APOE* genotype, were included as fixed effects and screened for significance.

Heritability estimation using GCTA

To estimate the proportion of phenotypic variance due to genetics, GCTA Version 1.13 is using genotype data for relationship generation and restricted maximum likelihood (REML) to fit the linear mixed model. A genetic relationship matrix (GRM), the kinship matrix, is created for each pair of individuals from the total SNP similarity using autosomal genotype data. The genetic similarity is not restricted to genotyped SNPs itself, but also includes unknown causal SNPs that are in linkage disequilibrium with the genotyped ones. To generate a data set of unrelated individuals, thus less than fourth degree relatives, one of a pair of individuals was removed when the genetic overlap account for more than 2,5%. The same covariates, gender, age, year of education and *APOE* genotype, as for the family-based approach were included as fixed effects.

Bivariate genetic and environmental correlation estimation

Bivariate genetic and environmental correlation was estimated using SOLAR v.6.6.2 package for quantitative phenotype pairs if univariate heritability resulted in moderate to high heritability in the ASPFS. Genetic correlation is the component of total correlation that is due to shared genetic effects whereas the environmental correlation is derived from the environmental factors influencing both phenotypes. Similar to the univariate heritability estimation the same covariates were included.

3 Results

Genotyping

Genotyping using the Affymetrix Genome-Wide Human SNP Array 6.0 revealed 905 525 SNPs for 837 ASPS subjects. After filtering 536 954 SNPs remained in the sample set for heritability estimation using the GCTA software tool.

Heritability Estimation

Only individuals having complete data in each subtest were included in the heritability estimation for tested cognitive phenotypes using SOLAR, resulting in a total of 366 ASPFS samples. **Table 3** shows univariate heritability estimation results using SOLAR software. Three of four domains, memory, executive function and fine motor skills, revealed a moderate to high heritability. The domain for attention and speed showed low heritability. Low p-values for age indicated a significant influence in the variability of all tested cognitive abilities. Gender was significant in the domains of attention and speed and fine motor skills, but was not associated with memory and executive function as well as neuropsychological summary. The years of education were significant in the neuropsychological summary as well as in three subdomains, memory, executive function and fine motor skills. The *APOE* genotype was not associated in any cognitive domain and not in the neuropsychological summary.

ASPFS – Univariate Heritability Estimation							
				p-values for potential covariates			
	h^2	(SE)	*p*	age	gender	education	*APOE*
Neuropsychological Summary	0.62	(0.15)	1.07E-05	1.35E-40	4.07E-01	5.68E-10	8.17E-01
Memory	0.60	(0.14)	1.68E-05	1.23E-07	7.58E-01	5.22E-05	8.29E-01
Executive Function	0.41	(0.15)	3.49E-03	3.29E-07	6.50E-01	7.61E-07	6.37E-01
Attention and Speed	0.10	(0.14)	2.21E-01	9.62E-18	5.93E-04	8.62E-01	4.67E-01
Fine Motor Skills	0.59	(0.14)	2.14E-05	1.16E-32	2.02E-07	1.09E-05	1.31E-01

Table 3. Univariate heritability estimates for cognitive phenotypes of ASPFS samples using SOLAR software.

The heritability estimation presented in **Table 4** using GCTA included 479 individuals having complete data for each measurement used for the determination of cognitive abilities. After application of the kinship coefficient cut-off of 2.5 % all individuals remained in the analysis. Common SNPs and SNPs in linkage disequilibrium accounted for low to moderate heritability in all cognitive domains and in the neuropsychological summary, except the domain for attention and speed which was found to be high heritable.

ASPS – Univariate Heritability Estimation

	h^2	(SE)	p-value
Neuropsychological Summary	0,20	0,74	4,00E-01
Memory	0,11	0,74	4,00E-01
Executive Function	0,23	0,67	4,00E-01
Attention and Speed	0,61	0,74	2,00E-01
Fine Motor Skills	0,29	0,70	3,00E-01

Table 4. Univariate heritability estimates for cognitive phenotypes of ASPS ssubjects using GCTA software.

Bivariate estimation of genetic and environmental correlation for heritable cognitive abilities in the ASPFS is shown in **Table 5**. Low to moderate genetic and environmental correlation was observed in the domains. Memory showed strong correlation to executive function and moderate correlation to fine motor skills indicating for shared genetic factors. Executive function and fine motor skills were not genetically correlated. Memory and the executive function as well as in combination with fine motor skills showed low environmental correlation. Moderate environmental correlation was observed for executive function and fine motor skills.

	Memory	Executive Function	Fine Motor Skills
Memory		0.53 (0.18)	0.41 (0.17)
Executive Function	0.20 (0.19)		0.00 (0.24)
Fine Motor Skills	0.14 (0.23)	0.37 (0.18)	

Table 5. Bivariate analysis of heritable neuropsychological abilities in the ASPFS using SOLAR software. Correlation estimation of pairs of phenotypes and corresponding standard deviation () for genetic correlation above and environmental correlation below the diagonal.

4 Discussion

Most of the recent studies are focused on finding genetic loci in diseased samples like Alzheimer's disease [33, 34]. This study represents the findings of the heritability estimation of non-demented individuals for cognitive traits in the ASPFS, a family-based study, compared to the ASPS, a population-based study, with the inducement to detect possible heritable cognitive phenotypes in the normal aged population and to further examine probable contributing genetic factors in future.

The univariate family-based estimates for the domains of memory, executive function and attention are in line with those found in a family-based study [16]. Different study settings included the mean age and the neuropsychological measurement as well as lower sample size. A study examining the heritability of executive function comprising more than 700 individual teenage twins showed estimates of 10 to 42 % in subtests of the Wisconsin Card Sorting Test. This is comparable to the results of the executive function found in the ASPFS where parts of the same neuropsychological test were used. Compared to the high heritable result of fine motor skills of 59 % in the ASPFS measured by the Perdue Pegboard Test a former study on heritable cognitive abilities in families with members diagnosed with schizophrenia were found to be moderate with estimated heritability of 35 % [35]. The age in this study ranged from 13 to 56 years. The higher estimate might be due to the older aged individuals in the ASPFS.

Inclusion of the demographic covariates age, gender, years of education and *APOE* genotype was applied to avoid confounding effects. Significance for covariates was itemized in the ASPFS only. Age was found to significantly affect all domains and the higher level of neuropsychological summary. Gender is not affecting the cognitive abilities of memory, executive function and the overall neuropsychological summary. Whereas the domains attention and speed and fine motor skills has shown to be affected by gender. A study on gender difference and cognitive performance revealed no association between gender and cognition in a small sample size [36]. This study might be to underpowered to find an association with gender. The examination of the ASPFS might be too

underpowered to find also gender differences in the other domains. A further gender-separated examination of attention and speed and fine motor skills is necessary for further interpretations. Education was significant in all domains and in the higher level, but not in fine motor skills. This leads to the suggestion that the educational attainment might influence the domains in the way of cognitive reserve. The genotype homozygous for allele 3 in *APOE* was in both studies the most common one, all other genotypes were barely present. This lead to the suggestion the *APOE* genotype distribution would need more samples carrying also the other *APOE* variations to show an effect on cognitive functioning.

As expected large difference in univariate heritability estimates were found when comparing the results of the two studies. The low to moderate heritability estimates obtained from the analysis using genotyped SNPs are faced with the estimates from pedigree data with moderate to high values, except the domain for attention and speed.

Those differences are accounted to the provided data input. As described earlier a limitation of heritability estimation using GCTA is that only common SNPs and additionally the SNPs in linkage disequilibrium are present. Those detectable variants applied on high-density SNP arrays show minor allele frequencies of more than 1 % in the population. Consequently rare SNPs are likely to be missed in the analysis and only additive genetic effects of common variants are included. Pedigree-based heritability estimates are made due to common and inherited rare SNPs as well as non-additive effects included in the pedigree information and are consequently higher than those estimations of the ASPS. In order to the divergent results one can conclude that almost half of the genetic variance contributing to the phenotypic variance is due to common variants, but non-additive genetic factors and rare SNPs are important as well [37, 38].

The high heritability for attention and speed estimated by GCTA was ascribed to chance, an estimate of around 5 % was expected. A replication in a larger sample for this domain is required for a definite result and further interpretation.

Strong genetic correlation was found in the ASPFS for memory and executive function with 53 %, this is similar to detected correlation in a former cited study [16]. Moderate correlation, 41 %, was found for the domains of memory and fine motors skills. This indicates that the pairs of domains have some of genetic factors in common. This is not unusual in complex organism and does include genes as well as non-coding DNA in their cascading effects [39]. Non-shared genetics were observed when correlating executive function and fine motor skills. The results of the bivariate analysis give rise to examine the probable shared profound genetics for those cognitive phenotypes.

Environmental correlation was estimated to be 20 % for memory and executive function and 14 % for memory and fine motor skills. In contrast to the absent genetic correlation of executive function and fine motor skills an environmental correlation of 37 % was observed. Even if the environmental effects are low to moderate, it seems like other environmental factors than those included as covariates, for example years of education, are affecting the variance of the cognitive traits. A study describing the influence of physical exercise to memory performance indicates for interference [40]. Here, an increase of the hippocampal volume due to aerobic exercise in the elderly and as a result influence on the spatial memory was observed. Additional collection and including more environmental data in further analysis is necessary to examine environmental factors contributing to cognitive abilities.

In sum, univariate heritability estimates of cognitive abilities, except for the attention and speed domain, computed with common SNPs accounted for about half of the family-based approach. Hence, further approaches using common SNPs are able to detect genetic factors associated with cognitive abilities in an adequate large sample size partially. Rare variants are part of the missing heritability and might include variants contributing to the phenotypic variance of cognitive abilities. The bivariate approach give rise to detect generalist genes included in the pathway from genes to brain function to cognitive performance. Future studies are needed to find genetic and environmental factors contributing to cognitive abilities to further identify causal genes in the pathology of disorders including cognitive decline.

5 References

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