Genetic variation underlying cognition and its relation with neurological outcomes

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ABSTRACT

Background General cognition in adults shows variation due to brain and cognitive reserve, and degenerative components. A recent genome-wide association study identified genetic variants for general cognitive function in 148 independent loci. The relation of these variants with cognitive decline, the incidence of dementia, parkinsonism and stroke, and brain imaging is still unknown. Here, we aimed to elucidate these associations.

Methods This study was conducted within the population-based Rotterdam Study (mean age 65.3±9.9 years, 58.0% women). We used lead variants for general cognitive function to construct a polygenic score (PGS). Additionally, we excluded genetic variants previously associated with intracranial volume and educational attainment at multiple significance thresholds to eliminate the brain and cognitive reserve component.

Results A higher PGS including all lead variants (N=170) was related to a cognitive decline, higher educational attainment and larger intracranial volume. No association was found with daily functioning, or the incidence of dementia, parkinsonism or stroke. Excluding genetic variants associated with the cognitive and brain reserve component caused an attenuation of nearly all associations found.

Conclusion This study suggests that genetic variants associated with general cognitive function represent both reserve and degenerative components of cognitive performance.

INTRODUCTION

General cognitive function represents the ability to perform tasks across different cognitive domains. The development of the nervous system shapes an important part of the inter-individual variation in general cognitive function, with neurodegenerative processes increasingly contributing later in life.[1,2] The developmental component of cognition can be referred to as reserve and is subdivided into cognitive reserve and brain reserve. Cognitive reserve stands for a more efficient use of neural networks, or use of alternate networks, and is estimated with educational attainment; brain reserve represents a quantitative buffer of neural networks, of which intracranial volume is a marker.[3] As such, general cognition is a mixed construct consisting of both reserve and degenerative components[1], that has relevance for clinical outcomes with an important cognitive component, such as daily functioning, dementia, parkinsonism, and stroke.

Recently, the highly polygenic architecture of general cognitive function was partly elucidated by the identification of genetic variants in 148 independent loci (Davies et al. in press) One approach to provide more insight into underlying pathways, is to unravel the relation of these variants with markers of both reserve and degenerative components of general cognitive function. The association of these variants with specific clinical outcomes linked to accelerated cognitive decline could additionally contribute to understanding these pathways.

Brain changes may be a plausible biological substrate to explain possible underlying pathways between these genetic variants and cognitive performance, and subsequent clinical outcomes. Structural brain magnetic resonance imaging (MRI), including diffusion-MRI, is a suitable method for investigating brain changes that are important for cognition and related outcomes.[4-7]

In this population-based study, we aimed to elucidate the association of the recently identified genetic variants for general cognitive function with cognitive decline, measures of daily functioning, the risk of neurological disorders, and structural neuroimaging.

METHODS

Study population

This study was conducted within the framework of the Rotterdam Study, an ongoing population-based cohort study located in the Netherlands with the aim to investigate causes and determinants of diseases in the elderly.[8] This cohort was initiated in 1990 and extended in 2000 and 2006, with a total of 14926 participants aged 45 years and older who undergo examinations every three to four years. Assessment of dementia, parkinsonism including PD, and stroke has been performed since the start of the study.

In 2002, an extensive cognitive test battery was added to the core protocol. MRI scanning was implemented in the study protocol from 2005 onwards.[9] Out of 14926 subjects, genotyping was successfully performed in 11496 participants. **Figure 1** gives an overview of the selection of participants for the different analyses, presented in a flowchart. After application of the exclusion criteria, different subgroups of participants remained for different analyses (**Figure 1**). According to the Population Study Act Rotterdam Study, the Ministry of Health, Welfare and Sports of the Netherlands has given approval for the Rotterdam Study. All participants have given written informed consent.[8]

Genotyping

The Illumina 550K, 550K duo and 610 quad arrays were used for genotyping. Samples with a call rate below 97.5% were removed, as well as gender mismatches, excess autosomal heterozygosity, duplicates or family relations, ethnic outliers, variants with call rates lower than 95.0%, failing missingness test, Hardy-Weinberg equilibrium p-value smaller than 10^{-6} and allele frequencies smaller than 1%. Genotypes were imputed using MaCH/minimac software to the 1000 Genomes phase I version 3 reference panel.

Polygenic score

We made a polygenic score (PGS) for all participants using the 178 lead single nucleotide polymorphisms (SNPs) with the corresponding effect sizes as described by Davies *et al.*(in press) Variants that were not available in the reference panel and variants with an r^2<0.30 were excluded (N=7 and N=1, respectively). For the remaining genetic variants (N=170), the allele dosage was multiplied by the reported effect estimate. Subsequently, the weighted effects of all variants were added up and the resulting polygenic scores were standardized into Z-scores.

Furthermore, we aimed to differentiate the reserve component of general cognitive function from the degenerative effects. Therefore, we calculated additional PGSs where variants associated with educational attainment and intracranial volume were removed using multiple p-value thresholds. For each variant, we used the lowest p-value threshold for either educational attainment or intracranial volume. The p-values were extracted from the summary statistics of a GWAS on educational attainment performed in 120000 individuals, and a GWAS on intracranial volume performed in 32438 individuals.[10] For all 170 variants included in the abovementioned PGS, information was available in both the educational attainment and intracranial volume GWAS. The different p-value thresholds for the association with educational attainment and intracranial volume, with the corresponding number of variants that remained, and the explained variance of the G-factor in our dataset are shown in **Supplementary**

Table S1. When applying the strictest p-value threshold (p>0.05), 36 genetic variants remained.

Cognitive test battery

The Mini-Mental State Examination (MMSE) was assessed as a measure of global cognitive function. Additionally, cognitive function was assessed using multiple cognitive tests: the 15-word learning test (15-WLT), the Stroop test (consisting of reading, color naming and interference tasks, error-adjusted scores), the Letter-Digit Substitution Task (LDST), the Word Fluency Test (using animal categories) and the Purdue pegboard (PPB) task for the left hand, right hand and both hands.[2,11-14] A measure of general cognitive function ("G-factor") was obtained through principal component analysis on the delayed recall score of the 15-WLT, Stroop interference test, Letter-Digit Substitution Task, Word Fluency Task and the Purdue Pegboard test, as described previously.[2] The G-factor explained 53.4% and 51.9% of the variance in cognitive test scores in our population at baseline and follow-up visit, respectively. Z-scores were calculated in order to make comparable test results. Self-reported years of education was used as a measure of educational attainment.

Assessment of daily functioning

Two components of daily functioning were assessed: basic activities of daily living (BADL) and instrumental activities of daily living (IADL). The Dutch version of the Stanford Health Assessment Questionnaire was used to measure BADL[15] and IADL was measured using the Dutch version of the Instrumental Activities of Daily Living scale.[16] To prevent selective loss of data, IADL items scored as non-applicable were imputed using the variables age, sex, BADL scores and all other available IADL items. Both BADL and IADL scores were standardized into Z-scores. Lower scores correspond to better daily functioning.

Assessment of clinical outcomes

The assessment of dementia, parkinsonism (including PD) and stroke were previously described in detail.[17-19] In summary, history of these clinical outcomes was assessed during the baseline interview. Participants were screened at baseline and subsequent center visits for dementia with the MMSE and the Geriatric Mental Schedule organic level, and for signs of parkinsonism. Participants with a positive screening were further examined and were evaluated by a panel led by an experienced neurologist who made the definitive diagnosis. After enrollment, participants were continuously monitored for dementia, parkinsonism and stroke through automated linkage of the study database with files from general practitioners. Follow-up for parkinsonism (including

PD) was available until the 1st January 2015 and for dementia and stroke until the 1st January 2016.

MRI acquisition and processing

We performed a multi-sequence brain MRI scan on a 1.5 tesla research dedicated MRI scanner (GE Signa Excite). Imaging details are provided elsewhere.[26] In short, the scan protocol included a T1-weighted image, a T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence, a proton density weighted image and a spin echo echo planar diffusion weighted image for the diffusion-MRI. Scans were segmented into grey matter, white matter, white matter lesion volume, cerebrospinal fluid and background tissue.[20,21] We estimated supratentorial intracranial volume by summing total grey and white matter volume and cerebrospinal fluid.[20]

For the diffusion-MRI, three volumes were performed without diffusion weighting (b-value=0 s/mm^2, maximum b-value was 1000 s/mm^2). Diffusion tensors were computed using ExploreDTI to obtain fractional anisotropy (FA) and mean diffusivity (MD) in normal-appearing white matter voxels. We segmented fifteen white matter tracts using probabilistic tractography and atlas-based masking.[22] Tracts were grouped based on anatomic location or presumed function into brain stem tracts, projection tracts, association tracts, limbic system tracts and callosal tracts. Tract-specific FA and MD but also white matter volumes and white matter lesion volumes in specific tracts were obtained as previously described.[22]

In general, a lower FA and a higher MD are indicative of lower microstructural white matter integrity

Data analysis

Linear regression models were used to assess the associations between the PGS and cognitive function and daily functioning cross-sectionally and longitudinally by means of change in cognitive and daily functioning over time. Cox proportional hazard models were used to study the association between the PGS and the incidence of dementia, parkinsonism, and stroke. The proportional hazards assumption and linearity assumption were met. We used linear regression models to study the relation between the PGS and volumetric brain outcomes and white matter microstructural integrity. All models were adjusted for age and sex. Models assessing change in cognitive function or daily functioning over time were additionally adjusted for baseline measurements, and time between baseline and follow-up visit. Models that assessed the relation between the PGS and volumetric brain outcomes were adjusted for intracranial volume when the outcome was not intracranial volume, and additionally for white matter and white matter lesion volume in the analyses with white matter microstructural integrity. The abovementioned analyses were repeated for all genetic variants separately.

Since outcomes for the different analyses may be correlated, we used permutation testing in order to assess the number of independent outcomes for each subsection. Based on this information, we defined the multiple testing p-value thresholds for the different analyses, namely $p<0.0038$ for the cross-sectional and $p<0.0040$ for the longitudinal analyses of cognitive performance and daily functioning; $p<0.0101$ for the volumetric and global diffusion-MRI brain measures, and $p<0.0022$ for the tract-specific diffusion-MRI analyses; and $p<0.0129$ for the clinical outcomes. For the analyses of the genetic variants separately, we additionally used the Bonferroni correction for multiple testing, using the formula k/170 with k representing the p-value threshold as obtained by permutation testing. Analyses were performed using the IBM SPSS Statistics 21 and R 3.4.0 software.

RESULTS

Genotyping data was available for 11496 individuals, with a mean age of 65.3±9.9 years, of which 58.0% was women. A flowchart for the inclusion of participants in the different analyses is shown in **Figure 1**. An overview of the characteristics of the study population for the different analyses are shown in **Table 1**.

Table 1. Study characteristics*

Characteristic	Sample									
			Dementia		Parkinson's disease		Parkinsonism		Stroke	
	Cognition and ADL N=5262	Brain imaging N=3710	N=11070	n=1444	N=10588	n=126	N=10826	n=258	N=11391	n=1220
Age, years	64.0±9.1	64.0 (11.0)	64.8±9.5	72.0±8.0	64.6±9.4	69.2±8.7	64.9±9.7	70.7±8.8	65.1±9.8	70.4±8.7
Female, % (N)	57.4 (3022)	55.0 (2039)	57.6 (6376)	68.0 (982)	57.3 (6065)	46.8 (59)	57.4 (6219)	52.3 (135)	58.2 (6436)	58.9 (718)
Follow-up time, years	6.1±0.6	-	12.2±6.4	11.3±6.3	12.4±6.5	7.8±5.9	12.3±6.5	7.7±5.8	12.3±6.6	9.4±5.9

*Values are expressed in mean±standard deviation unless stated otherwise; N is the total number of people for whom this characteristic is assessed; n is the number of cases.
Abbreviations: activities of daily living (ADL).

Cognitive performance and daily functioning

Figure 2, panel A shows the association between the genetic variants for general cognitive function and cognitive performance, daily functioning and educational attainment. An increase in the PGS was significantly associated with a higher G-factor ($\beta=0.08$, $p=9.6x10^{-14}$), as well as with individual cognitive tests, except for the PPB test. The PGS was also significantly associated with more years of education ($\beta=0.29$,

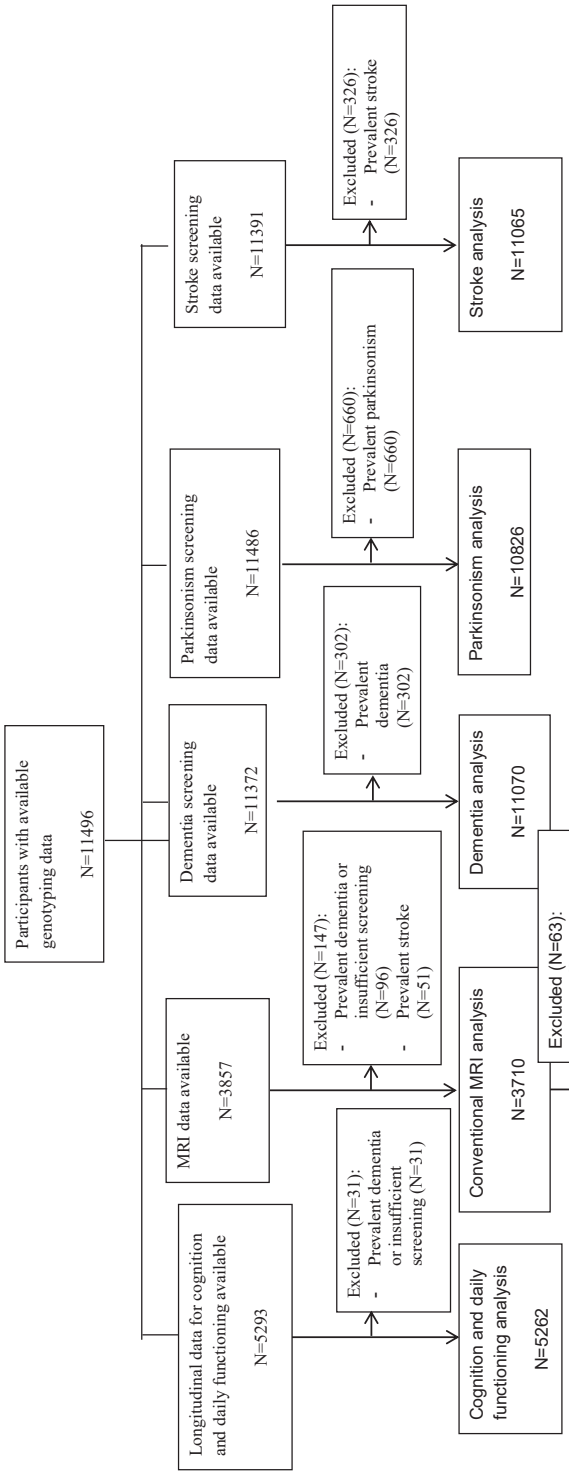

Figure 1. Flowchart presenting the in- and exclusions of participants in the different analyses.
Abbreviations: Magnetic Resonance Imaging (MRI).

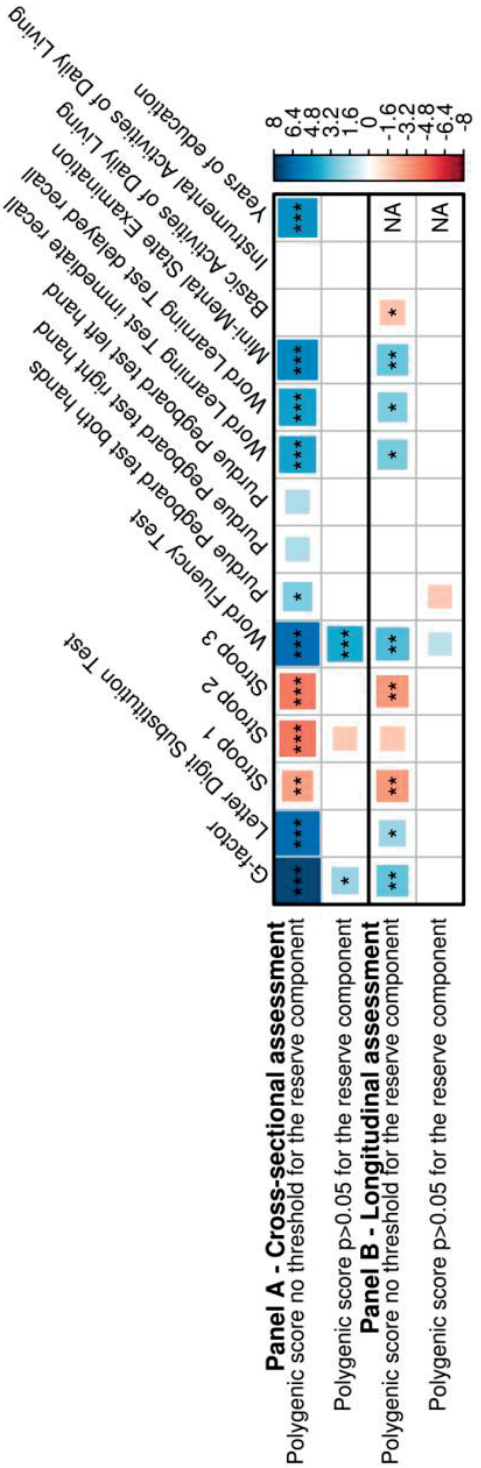

Figure 2. Association of G-factor genetic variants with (decline in) cognition and daily functioning, and educational attainment.

$p=5.2x10^{-7}$). No association with daily functioning was found. All associations attenuated after removing variants associated with the reserve component of cognition. No individual variant was significantly associated with any of the outcomes.

Figure 2, panel B shows that the PGS was associated with less decline in cognitive function ($\beta_{\Delta G\text{-factor}}=0.027$, $p=1.5x10^{-3}$). The PGS was also associated with less decline in BADL, although this was not significant after multiple comparison adjustments. Removing variants associated with educational attainment and intracranial volume resulted in an attenuation of the effects. In the single-variant analysis, no variant reached statistical significance for the association with cognitive decline or change in daily functioning.

Clinical outcomes

No significant association was found between the PGS and any of the clinical outcomes (**Figure 3**). Out of all 170 individual variants, none was significantly associated with the risk of one of dementia, parkinsonism or stroke. An increased risk for dementia was found after excluding variants associated with the reserve component at a $p>0.05$ threshold (hazard ratio 1.06, $p=0.040$), although this did not survive correction for

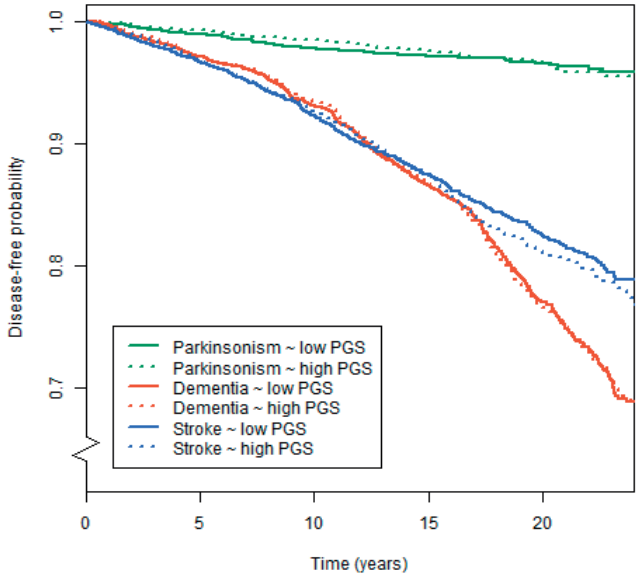

Figure 3. Polygenic scores for general cognitive function and disease-free probability for dementia, parkinsonism and stroke.

Kaplan-Meier curves presenting the association between low (i.e. below the median) and high (above the median) polygenic scores and the disease-free probability over time for dementia, parkinsonism, and stroke.

multiple testing.

Brain MRI markers

Figure 4 shows the results of the association of PGS with brain tissue volumes and global white matter microstructural integrity. We found that a higher PGS was significantly related to a larger intracranial volume (b=0.05, p=7.5x10^{-4}), but not with the other volumetric and global white matter microstructural integrity (**Figure 4**). At a nominal significance level, a higher PGS was associated with a higher FA in the medial lemniscus, and a lower MD in the inferior-fronto-occipital fasciculus and the posterior thalamic radiation, but this did not survive correction for multiple testing (data not shown). Removing genetic variants associated with the reserve component of cognition caused a weakening of the associations. No individual variant reached the significance threshold for the association with any of the brain imaging markers.

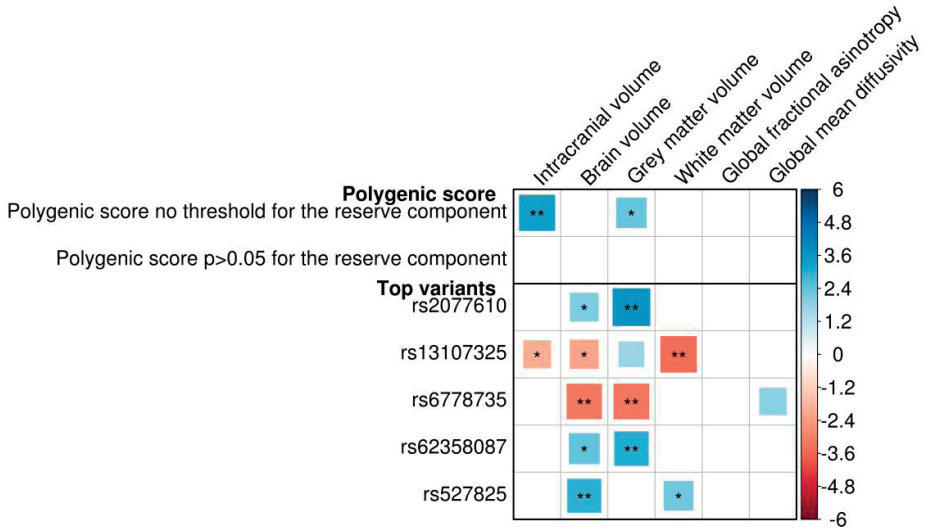

Figure 4. Association between genetic variants for general cognitive function and several brain imaging markers. Association between genetic variants for general cognitive function and both volumetric and global white matter microstructural integrity markers. Two polygenic scores are presented, one including all independent lead variants, and one only including variants with a p>0.05 for the association with the reserve component of cognition, which consists of cognitive reserve (educational attainment) and brain reserve (intracranial volume). Also, the five top genetic variants for the association with these brain imaging markers are presented. Positive associations depicted in blue correspond to a larger volume or a better white matter microstructural integrity. Larger blocks indicate higher t-values. Significance levels are indicated by asterisks: *p<0.05, nominally significant; **p<0.0101, adjusted for the number of independent traits as calculated through 10,000 permutations; ***p<5.9 x 10^{-5} (0.0101/170), additionally adjusted for the number of genetic variants.

Association between genetic variants for general cognitive function and cognitive function and daily functioning at one point in time, as well as years of education (panel A), and change in cognitive performance and daily functioning over time (panel B), adjusted for age and sex. Two polygenic scores are presented, one including all independent

lead variants, and one only including variants with a p>0.05 for the association with the reserve component of cognition, which consists of cognitive reserve (educational attainment) and brain reserve (intracranial volume). Larger blocks indicate higher t-values. Higher scores indicate better performance, except for the Stroop test, the Basic Activities of Daily Living and Instrumental Activities of Daily Living. Significance levels are indicated by asterisks: *p<0.05, nominally significant; **p<0.0038 (**Figure 2, panel A**) or p<0.0040 (**Figure 2, panel B**), adjusted for the number of independent traits as calculated through 10000 permutations; ***p<2.2 x 10^{-5} (**Figure 2, panel A**; 0.0038/170) or p<2.4 x 10^{-5} (**Figure 2, panel B**; 0.0040/170), additionally adjusted for the number of genetic variants.

DISCUSSION

In this population-based study among middle-aged and elderly persons, a PGS based on recently identified genetic variants for global cognition was associated with better global cognitive performance, better performance on individual cognitive tests, less cognitive decline, more years of education and a larger intracranial volume. We found no significant association with (decline in) daily functioning, the incidence of dementia, parkinsonism and stroke, or with any of the other global or white matter tract-specific brain imaging markers.

Cognitive decline is an important marker for the progression of neurodegenerative diseases.[23-25] Therefore, we related the genetic variants for global cognition to longitudinal assessments of cognitive and functional outcomes in our study and found that a PGS based on these genetic variants was associated with cognitive performance both cross-sectionally and longitudinally. This indicates that the genetic variants can partially explain inter- as well as intra-individual differences in cognition, and is supportive that a degenerative pathway underlies these identified genetic variants. However, no significant relation was found between the PGS and the risk of dementia, parkinsonism or stroke in this study. If anything, we saw a nominally significant association with the incidence of dementia, but the direction of effect was not as expected, i.e. a higher PGS associated with better cognitive function showed an increase in dementia risk. However, since this association did not survive correction for multiple testing, no strong conclusions should be drawn from this finding, and validation in other studies is needed. To our knowledge there are as yet no other studies that have investigated the association between these genetic variants and cinical outcomes. Previous studies did show an association between cognitive function and dementia, parkinsonism, Parkinson's disease (PD) and stroke, both before and after diagnosis.[7,24,26-29] Also, disease-specific genetic variants for these disorders have been associated with cognitive functioning.[30-33] This may indicate that cognitive decline as seen in abovementioned (prodromal) clinical

outcomes is mainly caused by disease-specific variants rather than variants for general cognitive function. In addition, it is possible that our study did not have enough power to detect a significant association with the incidence of the clinical outcomes due to the relatively small number of cases.

We also found that a higher PGS was related to a larger intracranial volume and higher educational attainment, both reflecting early brain growth and therefore suitable as markers for brain and cognitive reserve.[34,35] Brain reserve is partially determined by genetics[36-38] and is suggested to be protective against cognitive decline.[39]

Thus, our findings are supportive that the genetic variants act through both the reserve and neurodegenerative pathway. However due to absence of an association between the PGS and clinical outcomes, we attempted to enrich the degenerative component of the PGS by filtering out genetic variants hat are associated with intracranial volume and educational attainment. By applying this filter, nearly all associations for the different analyses were attenuated, supporting the suggestion that the genetic variants mainly represent the reserve component of cognitive performance. However, removing genetic variants associated with the reserve component may also eliminate degenerative components of the PGS if some variants are pleiotropic, possibly leading to an underpowered study for detecting an effect of the PGS. A more robust method would be to run a genome-wide association study (GWAS) with cognitive decline as an outcome instead of using cross-sectional measurements of cognitive performance, preferably in an elderly population since neurodegeneration mainly occurs later in life. However, longitudinal measurements such as those in the present study are only available in a fraction of the samples with cross-sectional assessments, which at present impedes GWAS discoveries for cognitive decline.

Strengths of this study are the population-based setting, the longitudinal assessment of cognitive function and daily functioning, the availability of structural brain imaging, and the long follow-up period for dementia, parkinsonism and stroke. We also need to consider limitations. It should be noted that the Rotterdam Study was part of the discovery cohort for the general cognitive function GWAS (Davies et al). However, since this was only a small proportion of the total sample size (2.0%), we do not expect that this influenced our findings to a large amount. This also leads to the limitation that the effect estimates in the summary statistics of the GWAS are based on the effect estimates of many different populations, and they may not be the correct estimates for the Dutch population as present in the Rotterdam Study.

In conclusion, we found that the PGS of general cognitive function was associated with cognitive performance, cognitive decline, intracranial volume and educational attainment, but not with daily functioning, neurological disorders, or the other brain imaging markers. Based on our results we postulate that the genetic variants identified for general cognitive function are acting mainly through the reserve pathway of cogni-

tion, but also affect the degenerative pathway. Further genetic studies of cognitive decline in elderly individuals can provide more insight into the degenerative pathway.

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Supplementary Table S1. P-value thresholds for the association with educational attainment and intracranial volume, and corresponding number of genetic variants remaining.

Threshold p-value for the association with educational attainment and intracranial volume	Number of variants remaining	Explained variance G-factor
No threshold	170	0,00656
$P>5e^{-8}$	149	0,00593
$P>1e^{-7}$	146	0,00607
$P>1e^{-6}$	144	0,00594
$P>1e^{-5}$	135	0,00507
$P>1e^{-4}$	121	0,00431
$P>1e^{-3}$	101	0,00299
P>0.01	70	0,00186
P>0.05	36	0,00062