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Grey matter heritability in family-based and population-based studies using voxel-based morphometry

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Abstract (250 words)

Background: The combination of genetics and imaging has improved our understanding of the brain through studies of aggregate measures obtained from high-resolution structural imaging. Voxel-wise analyses have the potential to provide more detailed information of genetic influences on the brain. Here we report a large-scale study of the heritability of grey matter at voxel resolution (1×1×1mm). **Methods**: Validated voxel-based morphometry (VBM) protocols were applied to process magnetic resonance imaging data of 3239 unrelated subjects from a population-based study and 491 subjects from two family-based studies. Genome-wide genetic data was used to estimate voxel-wise gray matter heritability of the unrelated subjects and pedigree-structure was used to estimate heritability in families. We subsequently associated two genetic variants, known to be linked with subcortical brain volume, with most heritable voxels to determine **i**f this would enhance their association signals. **Results**: Voxels significantly heritable in both estimates mapped to subcortical structures, but also voxels in the language areas of the left hemisphere were found significantly heritable. When comparing regional patterns of heritability, family-based estimates were higher than population-based estimates. However, regional consistency of the heritability measures across study designs was high (Pearson's correlation coefficient=0.73, p=2.6×10^{-13}). We further show enhancement of the association signal of two previously discovered genetic loci with subcortical volume by using only the most heritable voxels. **Conclusion**: Grey matter voxel-wise heritability can be reliably estimated with different methods. Combining heritability estimates from multiple studies is feasible to construct reliable heritability maps of grey matter voxels.

1. Introduction

The human brain shows large inter-individual variation, which could be explained by genetic and environmental influences. Studying these influences is essential in better understanding brain structure and function. The degree to which genetics explains phenotypic variation, in other words heritability, depends on many factors: the actual genetic contribution to the trait, environmental effects, measurement error, study design and sample characteristics [Visscher, et al., 2008; Visscher, et al., 2006; Yang, et al., 2010]. Recently an overview was published of fifty years of worldwide heritability research in twins encompassing thousands of traits, showing heritability studies are highly informative on how large the genetic contribution to a trait is [Polderman, et al., 2015]. Heritability studies could aid future genetic research to focus on particular regions of interest in the brain. For example, large scale genetic studies of brain structures with the highest heritability typically yield the most findings [Hibar, et al., 2015]. When studying the multitude of measures of voxel based magnetic resonance imaging (MRI), limiting genetic studies to the most heritable traits could be feasible in light of multiple testing. Recent studies have focused on heritability of detailed MRI measures at a voxel level [Brouwer, et al., 2010; Ganjgahi, et al., 2015; Jahanshad, et al., 2013; Jahanshad, et al., 2010; Kochunov, et al., 2016; Kochunov, et al., 2010; Kochunov, et al., 2015]. Different study designs showed comparably high estimates for white matter tract heritability in twin pairs [Brouwer, et al., 2010; Kochunov, et al., 2010], sib-pairs [Jahanshad, et al., 2010] and extended pedigrees (heritability = 50-90%) [Ganjgahi, et al., 2015]. The heritability of grey matter was studied by voxel-based morphometry (VBM) previously [Hulshoff Pol, et al., 2006; Peper, et al., 2009; Thompson, et al., 2001], but the studies were relatively small and relatively large voxels were studied. Moreover, heritability of grey matter VBM has not been estimated in population-based studies.

Here, we perform a large multi-site study to estimate the voxel-wise heritability of grey matter. We calculate pedigree-based heritability in two family-based studies and heritability based on genome-

wide genetic data in a large population-based study of unrelated subjects. Using these approaches, we created two grey matter heritability maps and described which regions contain significantly heritable voxels in both designs. We furthermore estimated overall regional consistency of the heritability measures across study designs and explored if usage of our heritability maps could potentially enhance association signals of two genetic variations, previously discovered by genome-wide association studies [Bis, et al., 2012; Hibar, et al., 2015; Stein, et al., 2012].

2. Methods

Study subjects and imaging protocol

Rotterdam Study – The Rotterdam Study is a population-based cohort study among inhabitants of a district of Rotterdam (Ommoord), The Netherlands, and aims to examine the determinants of disease and health in the elderly with a focus on neurogeriatric, cardiovascular, bone, and eye disease [Hofman, et al., 2015]. In 1990 to 1993, 7983 persons participated and were re-examined every 3 to 4 years (RS-I). In 2000 to 2001 the cohort was expanded by 3011 persons who had not yet been part of the Rotterdam Study (RS-II). All participants had DNA extracted from blood at their first visit. In 2006-2008 a second expansion (RS-III) of 3,932 persons aged 45 and over was realized. Genotyping was performed at the Human Genotyping Facility, Genetic Laboratory Department of Internal Medicine, Erasmus MC, Rotterdam. Genotypes were imputed to the 1000 genomes phase I version 3 reference panel, using standard methods and software [Willer, et al., 2008]. From 2005 onwards MRI is part of the core protocol of the Rotterdam study [Ikram, et al., 2015]. For this study a total of 4071 unique study participants had both MRI and genetic data and were available for analysis. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Erasmus Rucphen Family (ERF) – The ERF study is a family-based cohort study in a genetically isolated population from a community in the South-West of the Netherlands (Rucphen municipality) including 3000 participants. Participants are all descendants of a limited number of founders living in the 19th century, and all of Caucasian European descent. Extensive genealogical data is available for this population. The study population is described in detail elsewhere [Sayed-Tabatabaei, et al., 2005]. In a follow-up analysis, non-demented hypertensive (systolic blood pressure ≥ 160, diastolic blood pressure ≥ 100 or use of antihypertensive medication) subjects aged 55-75 years were included for a new battery of tests, including MRI scanning [Ibrahim-Verbaas, et al., 2012]. These 122 participants from the ERF were related to each other in one large pedigree. This large pedigree was split into multiple small pedigrees for heritability calculations (pedcut version 1.19 http://mga.bionet.nsc.ru/soft/). Participants related to each other in 27 families with in total 880 relatives. The average size of the pedigrees was 32.6 relatives (range 20-44) with on average 4.5 participants with MRI per family. All participants gave informed consent to participate in the study and to obtain information from their treating physicians. The study was approved by the medical ethics committee at Erasmus MC University Medical Center, Rotterdam, The Netherlands.

MRI scanning for ERF and the Rotterdam Study was done on the same 1.5 T MRI unit (GE Healthcare, Milwaukee, USA, Signa Excite software version 11×) fitted with a dedicated 8-channel head coil. The T1-weighted, proton density-weighted (PDw) and fluid-attenuated inversion recovery (FLAIR) sequences were used [Ikram, et al., 2015]. For the purpose of segmentation, the T1w scan is acquired in 3D at high in-plane resolution and with thin slices (voxel size < 1 mm3 [Ikram, et al., 2015].

Austrian Stroke Prevention Study (ASPS) – The ASPS study is a single-center, prospective follow-up study on the effects of vascular risk factors on brain structure and function in the normal elderly population of the city of Graz, Austria. The procedure of recruitment and diagnostic work-up of

study participants has been described previously [Schmidt, et al., 1999; Schmidt, et al., 1994]. Between 2006 and 2013 the study was extended for the Austrian Stroke Prevention Family Study (ASPS-Fam) [Seiler, et al., 2014]. Study participants of the ASPS and their first grade relatives were invited to enter ASPS-Fam. Inclusion criteria were no history of previous stroke or dementia and a normal neurological examination. In total 176 families connecting a total of 719 relatives, among which 369 were study participants with brain-MRI. The average size of the pedigrees was 4 (range 1-10) relatives with on average 2.4 participants with MRI per family. The diagnostic work-up was identical to the original study. The study protocol was approved by the ethics committee of the Medical University of Graz, Austria, and written and informed consent was obtained from all subjects. MRI scanning of the ASPS-Fam was done on a 3.0 T Tim Trio (Siemens, Erlangen). T1-MPRAGE 1×1×1mm was used for image processing [Seiler, et al., 2014].

Image processing

Prior to analysis, a number of pre-processing steps were performed. For multispectral image analysis, the different scans were spatially registered using rigid registration [Ikram, et al., 2015]. Subsequently, the brain was extracted from the scan. Hereto a manually segmented brain mask, which excludes cerebellum, eyes and skull, was non-rigidly registered to the T1-weighted image using Elastix [Ikram, et al., 2015]. Finally, scans were corrected for intensity non-uniformity using the N3 method; non-uniformity correction was carried out within the brain mask [Ikram, et al., 2015]. All T1-weighted images were segmented into supra-tentorial grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). For the Rotterdam Study and ERF, we used a previously described k-Nearest-Neighbor (kNN) algorithm, which was trained on six manually labeled atlases [Vrooman, et al., 2007]. For the ASPS-Fam study a Quantib BV tissue segmentation tool was applied (www.quantib.org). Quantib® software implements the same algorithm, which we then used for tissue segmentation in the Rotterdam Study and ERF. There are thus no methodological differences

between the methods, both of them based on kNN-based segmentation training on manually labeled subjects for segmenting GM, WM and CSF.

Voxel-based morphometry (VBM) was performed by the same optimized VBM protocol in all three studies [Good, et al., 2001] and previously described [Roshchupkin, et al., 2016a]. FSL software [Smith, et al., 2004] was used for VBM data processing. First, all GM density maps were non-linearly registered to the standard GM probability template. For this study we chose the MNI152 GM template (Montreal Neurological Institute) with a 1×1×1 mm voxel resolution [Fonov, et al., 2011]. The MNI152 standard-space T1-weighted average structural template image is derived from 152 structural images, which have been warped and averaged into the common MNI152 coordinate system after high-dimensional nonlinear registration. A spatial modulation procedure was used to avoid differences in absolute grey matter volume due to the registration. This involved multiplying voxel density values by the Jacobian determinants estimated during spatial normalization. To decrease signal to noise ratio, all images were smoothed using a 3 mm (FWHM 8 mm) isotropic Gaussian kernel. Thus all results are in MNI space. Brain regions were segmented using atlas-based segmentation based on the Hammer atlas [Hammers, et al., 2003]. The modulation step in the VBM pipeline preserves the volume of a particular tissue within a voxel. The multiplication of the voxel values in the segmented images by the Jacobian determinants derived from the spatial normalization step allows us to calculate volumes by aggregating voxels. In total we estimated heritability for 1,405,508 grey matter voxels in all three studies.

Reproducibility VBM measures

We investigated the test-retest reliability of the VBM measures in a subset of 83 persons who were scanned twice within 1-9 weeks. We quantified the reproducibility by calculating the intraclass correlation (ICC) of the gray matter density measures for every voxel (Online viewer, Supplementary Figure 1)[Shrout and Fleiss, 1979]

Heritability analysis

Population-based heritability estimates were calculated using Genome-wide Complex Trait Analysis (GCTA v1.24) [Yang, et al., 2011] (http://cnsgenomics.com/software/gcta/) in the population-based Rotterdam Study. GCTA implements REML (restricted maximum likelihood) analysis, this method compares genotypic similarity between individuals to their phenotypic similarity. **Formula's underlying the GCTA method to determine heritability estimates are described elsewhere [Yang, et al., 2010] and thoroughly explained in a commentary by the authors [Visscher, et al., 2010]**. The 1000 Genomes imputed genotypes (Imputation quality (Rsq) > 0.5 and minor allele frequency (MAF) > 0.01) were used to create a genetic relationship matrix (GRM) in GCTA [Adams, et al., 2016]. **The power of GCTA analysis is determined by pair-wise genetic relationships in the studied population [Visscher, et al., 2010; Yang, et al., 2010]. Therefore the three cohorts of the Rotterdam study were combined and analyzed as one in the voxel-wise heritability analysis.** Pairwise genetic relatedness between all individuals (N=4071) was calculated and for pairs with more than 0.02 genotype similarity [Adams, et al., 2016] one person was removed ($N_{removed}$ = 832). REML analysis was then performed in the remaining 3239 unrelated subjects using the GRM correcting for age and sex. All grey matter heritability was estimated once**.**

Family-based heritability was estimated using maximum-likelihood variance components methods implemented in the SOLAR (version 6.6.2) [Almasy and Blangero, 1998] software. Formulas for the calculation of heritability estimates are described in detail elsewhere [Almasy and Blangero, 1998]. Briefly, the algorithms in SOLAR employ maximum likelihood variance decomposition methods. The covariance matrix Ω for a pedigree of individuals is given by:

$$\Omega = 2 \cdot \Phi \cdot \sigma_g^2 + I \cdot \sigma_e^2$$

where σ^2_g is the genetic variance due to the additive genetic factors, Φ is the kinship matrix representing the pair-wise kinship coefficients among all individuals, σ^2_e is the variance due to individual-specific environmental effects, and I is an identity matrix (under the assumption that all environmental effects are uncorrelated among family members). Narrow sense heritability is defined as the fraction of phenotypic variance σ^2_P attributable to additive genetic factors:

$$h^2 = \frac{\sigma_g^2}{\sigma_P^2}.$$

The variance parameters are estimated by comparing the observed phenotypic covariance matrix with the covariance matrix predicted by kinship (Almasy and Blangero, 1998). Significance of heritability is tested by comparing the likelihood of the model in which σ^2_g is constrained to zero with that of a model in which σ^2_g is estimated. Twice the difference between the $\log_e$ likelihoods of these models yields a test statistic, which is asymptotically distributed as a ½:½ mixture of a χ^2 variable with 1 degree-of-freedom and a point mass at zero. If the algorithm converges SOLAR outputs the heritability value, the significance value (p), and the standard error for each voxel [Almasy and Blangero, 1998; Kochunov, et al., 2015].

ERF study and ASPS-Fam were not jointly analysed because ERF subjects were scanned on a 1.5T MRI and ASPS-Fam subjects on a 3.0T MRI. Instead inverse variance meta-analysis using heritability and heritability standard errors was performed in METAL [Willer, et al., 2010] to boost power and improve stability of heritability estimates [Jahanshad, et al., 2013]. Heritability estimates were calculated in both studies with age and sex as covariates. Variance component methods implemented in SOLAR are vulnerable for inflation if phenotypes have a leptokurtic to distribution. Therefore we applied inverse normal transformations in SOLAR to all voxels, but some voxels still violated the distribution of too high residual kurtosis (kurtosis >0.9) and were therefore excluded [Blangero, et al., 2001]. Non converging heritability estimates of 0 without standard errors were also

excluded from the meta-analysis. **In the family-based studies some voxels had valid p-values and a heritability of 1, but missing standard errors. These voxels were located in the middle of voxel-clusters with high heritability (online viewer reference) (close to 1). Therefore standard errors for such voxels were imputed to retain these voxels for meta-analysis. This resulted in imputation of the standard error for 6.4% of voxels in the ERF study and a negligible percentage of voxels in ASPS-Fam (<0.001%).**

Enhancement of association signal

We explored whether voxel heritability information could enhance the association of genetic variants with brain structures. The genetic variants most significantly associated with hippocampal volume (rs77956314 on 12q24.22, near the gene *HRK*) and putamen volume (rs945270 on 14q22.3, downstream of the gene *KTN1*) were selected from a recently published genome-wide association study on subcortical structures [Hibar, et al., 2015]. To select the most heritable voxels in the hippocampus and putamen, we ordered them using three approaches. First, we ranked the voxels from low to high family-based heritability estimates. Second, we ranked them from low to high population-based heritability estimates. In the third approach we summed the ranks obtained from both the family- and population-based estimates and used the sum of the ranks to prioritize the voxels. Using these three approaches we excluded the voxels in a step-wise manner by removing the 5% least heritable voxels. For each step we computed the volume by summing the values of the remaining voxels. As a voxel represents grey matter density in 1 mm^3, the sum of voxels gives the volume of grey matter. We determined the association of the two genetic variants in an additive model with the volumes in linear regression analyses (adjusted for age, sex, and the first three principal components) and compared this to association of the volume derived from all voxels mapped to the structure (i.e. the total VBM-volume of the hippocampus or putamen). The p-value of the association of the genetic variants with the subsets of voxels divided by the p-value of the association of the genetic variants with the total VBM-volume was calculated to measure change in

the strength of the association. Genetic effects were calculated in the three cohorts of the Rotterdam study separately (RS-I = 844, RS-II = 1003, RS-III = 2190) and were combined using an inverse variance weighted meta-analysis in METAL [Willer, et al., 2010].

Statistical analysis

Descriptive statistics were compared using one-way ANOVA and chi-squared tests. **To correct for multiple comparisons we applied FDR p-value thresholds [Benjamini and Hochberg, 1995] for both population and family heritability separately to declare which voxels are significantly heritable.**

3. Results

Population characteristics

Characteristics of the study population are shown in **Table 1.** The spread of the age of subjects in the ERF study (age range 55-76) was smaller compared to ASPS-Fam (38-86) and the Rotterdam Study (46-98) due to the fact that inclusion criteria for scanning was restricted to midlife **(Table 1)**. However, the average age at the time of MRI-scanning of the cohorts was very similar, ranging from 64.3 (± 4.5) years in the ERF study, 64.9 (± 10.7) years in ASPS and 64.9 (± 10.7) in the Rotterdam Study ($p = 0.86$). The percentage of women was 52.5% in ERF, 60.4% in ASPS-Fam and 55.3% in the Rotterdam study, these differences were non-significant ($p = 0.13$) (**Table 1**).

Heritability estimates

In total 454,184 (33.3% of all voxels) were FDR-significant in the family-based estimates. Mean heritability of significant voxels was 0.44 ± 0.12 SD **(all voxels 0.29 ± 0.17 SD)**, with heritability estimates ranging from 0.23 to 1. In total 68,616 (4.9% of all voxels) were FDR-significant in the population-based estimates. Mean heritability of the significant voxels was 0.34 ± 0.04 SD **(all voxels 0.11 ± 0.10)**, with heritability estimates ranging from 0.25 to 0.56. We found heritability of 44,349

voxels (3.2% of all voxels) to be FDR significant in the family- as well as the population-based heritability estimates**.** These significantly heritable voxels were clustered, mostly within subcortical brain structures (**Figure 1). Table 2** shows the percentage of voxels that were significantly heritable of the total of voxels in a structure in both estimates, as well as the average regional heritability, considering all voxel-wise heritability estimates. Highest percentage of significantly heritable in both estimates voxels were located in the caudate nucleus (right 72.4% and left 68.6%) followed by the putamen (right 57.5% and left 32.6%). Other subcortical structures with a large percentage of significantly heritable voxels were; left pallidum (32.2%), left nucleus accumbens (29.7%), right pallidum (28.5%), left amygdala (21.4%), left hippocampus (17.9%), left thalamus (14.4%), right amygdala (12.8%) and the right insula (11.4%). Apart from the subcortical structures, parts of the right lateral occipitotemporal gyrus (gyrus fusiformis) (10.4%), left straight gyrus (gyrus rectus) (10.4%), left subcallosal area (8.0%) and the left lingual gyrus (7.9%) harbored a proportion significantly heritable voxels (**Table 2 and Figure 1**).

When comparing regional heritability, estimates calculated in families was always higher than the population-based estimates ($p<0.001$) **(Figure 2 A)** and the difference in heritability between family-based estimates and population-based estimates was relatively stable (mean difference of regional heritability = 0.21 ± 0.08) **(Table 2)**. Therefore, the regional heritability pattern of the family-based estimates significantly predicted the regional pattern of heritability in the population-based study (Pearson's correlation coefficient = 0.73, $p = 2.6 \times 10^{-13}$) (**Figure 2 B**).

Enhancement of association signal

We explored if applying our heritability map could enhance the statistical association signal of previously discovered genome-wide significant loci. As expected the T-allele of rs77956314 (*HRK*) associated with a smaller total volume of the hippocampus ($p = 5.1 \times 10^{-7}$) and the C-allele of rs945270 (*KTN1*) significantly associated with larger total volume of the putamen ($p = 4.3 \times 10^{-3}$).

When excluding the less heritable voxels the average heritability in the remaining voxels increased **(Figure 3 A and 3 B)**. **With rising average heritability we observed a gradual decrease in p-values (Figure 3C), and consequently a more significant association of HRK with the more heritable part of the hippocampus.** The maximum enrichment of association was reached when the 10% most significantly heritable voxels when combining heritability information from family-based and population-based studies was used. This increase corresponds to a 95.9 times more significant association, as the *p*-value decreased from $p=5.1 \times 10^{-7}$ to $p = 5.4 \times 10^{-9}$. **Using only the family-based estimates the association was 12.9 times more significant**. **A less substantial decrease in p-value was observed for the association of KTN1 with the more heritable part of the putamen (Figure 3 D).** The p-value decreased when restricting to voxels that belong to the 25% most heritable voxels from the only the family-based study. This corresponds to a 5.5 times more significant association (*p*-value decrease from $p = 4.3 \times 10^{-3}$ to $p = 7.9 \times 10^{-4}$).

Discussion

In this study we presented grey matter voxel heritability maps at resolution of 1×1×1 mm from population- and family-based studies. First we found that clusters of voxels that are significantly heritable in family-based heritability estimates as well as in an unrelated population-based study are predominantly located in subcortical regions. Second, when comparing the overall regional patterns of voxel-wise heritability the family-based estimates were always higher compared to population-based estimates and predicted the population-based heritability estimates. Lastly, we showed that the heritability estimates from our studies could be used to enhance the association signal of two genetic variants with subcortical volumes.

Voxels with significant heritability formed clusters within mainly the subcortical structures. This is in line with the findings of previous studies that the volumes of subcortical structure are among the most heritable in the brain [Blokland, et al., 2012]. There are multiple explanations for this

277 consistent finding. First, subcortical structures probably are under tight genetic control as they exert
278 vital functions within the brain. **The percentage of significantly heritable voxels was relatively low**
279 **in the frontal and parietal lobes. Although intra-individual measurability was high throughout the**
280 **brain (Supplementary Figure 1), intra-individual differences in cortical folding patterns could**
281 **explain the lower heritability in frontal and parietal regions. These might give a reliable**
282 **measurability of the voxels, while it makes comparisons of voxel values between individuals less**
283 **meaningful, thus yielding a lower heritability compared with the subcortical structures**. Finally,
284 environmental effects could have a larger effect on cortical grey matter compared to subcortical
285 structures. **As the effects of non-genetic factors (e.g. lifestyle factors) accumulate over an**
286 **individual's lifetime, the heritability of total brain volume and brain structures volume was found**
287 **to reduce in adulthood up until old age [Batouli, et al., 2014] in line with the accumulation of**
288 **environmental influences over age. Their reported maximum age was 70 years. We studied**
289 **relatively old participants (~65 years), therefore study participants might have reduced estimated**
290 **heritability because of their older age**

291 Apart from the subcortical structures, we found three cortical regions in the left hemisphere, the
292 dominant hemisphere in over 95% of individuals, involved in speech production and word processing
293 to have more than 5% significant voxels; the subcallosal area (also called Broca area), central part of
294 the superior temporal gyrus (contains Wernicke's area) and the lingual gyrus. Moreover, their right
295 counterparts contained less significant voxels compared to the left side. Language skills[Gayan and
296 Olson, 1999] and brain networks [Budisavljevic, et al., 2015] are thought to be under tight genetic
297 control and the left hemisphere language areas have been found more heritable than the right
298 hemisphere before [Thompson, et al., 2001]. Regions with significant heritability could in theory be
299 connected by white matter connections, which in turn then also are under high genetic control,
300 suggesting a common genetic architecture. In a recent report evidence for this theory was found
301 [Shen, et al., 2016]. Cortical thickness in some regions with high heritability, were connected by

heritable white matter connections. These connections and the cortical regions were anatomically distant but showed significant genetically correlation [Shen, et al., 2016].

We found a relatively stable difference in the regional patterns of the total additive genetic heritability. The heritability calculated from familial relations was always higher than the total additive variance explained by all autosomal variants calculated in unrelated subjects. This known difference between family and population-based heritability estimates has been extensively described [Zuk, et al., 2012; Zuk, et al., 2014]. The difference can in part be explained by overestimation of heritability in families due to sharing of environmental factors within the family. These factors are interpreted as genetic effects and cause the overestimation of heritability in twin and nuclear family studies [Koran, et al., 2014]. Subjects in multi-generational families share less environmental factors. Therefore multi-generational families, as ASPS-Fam and especially the ERF study, are more likely to yield an unbiased estimate of heritability. However, we assumed that all environmental factors affecting brain voxel volume are uncorrelated among family members (unique environmental effects) therefore some unassessed common environmental effects might be causing the higher heritability in our family-based estimates. At the same time an underestimation of the heritability calculated from genetic data in unrelated populations could occur because of an incomplete coverage of the causal variants and exclusion of rare variants. We used imputed data to increase coverage of the causal variants. Imputed data provide a much denser coverage of the genome than only genotyped variants, but we did exclude rare variants (MAF<0.01) which may in part be responsible for some missing heritability.

The overall regional patterns of heritability from families strongly predicted the population-based heritability. This suggests that the regional pattern of variance explained by additive genetic effects is similar across populations, despite different ways to measure heritability, study design and scanner types. On the website (http://www.imagene.nl/heritability) both the population-based estimates and the family-based estimates can be viewed separately and can be downloaded.

Combining current maps with results from other studies will further increase accuracy of the heritability estimates.

Heritability in genetic studies

Within the putamen and hippocampus we observed highly heritable clusters of grey matter voxels alternating with parts of the subcortical structures that were less heritable. Differences in heritability within structures might be due to technical limitations (e.g. voxels that are difficult to measure) or due to genetic or functional correlations. We hypothesized that studying the genetics of only highly heritable voxels could enhance signals in imaging genetics, either through reducing signal to noise ratio or through studying a more genetically homogeneous trait. We picked two genetic variants with a proven and strongly replicated biological effect, identified through genome-wide association studies, on the subcortical structure volume (hippocampus, putamen) to explore if enhancement was possible [Hibar, et al., 2015]. We show enhancement of the statistical signal of almost hundred-fold for the association of *HRK* (rs77956314) with hippocampal volume and a five-fold increase for the association of *KTN1* (rs945270) with putamen volume. **Based on Figure 3 we can deduct that for future genetic studies in both examples a maximum power for association analyses was observed using voxels with a heritability over ~0.3 from the population-based heritability estimates and a heritability over ~0.7 from family-based heritability estimates. Despite these encouraging results there are limitations of our analysis. First, we only tested two genetic variants in two subcortical structures.** While we expect that the increased signal of genetic variants with more heritable voxels will not be limited to the two variants tested in current study, future studies applying this method should be performed to determine whether this truly is the case. Second, we calculated heritability estimates and genetic association of *HRK* and *KTN1* variants with voxels in the same subjects of the Rotterdam Study. As voxels with a large (technical) measurement error have lower heritability and therefore were excluded first in our analysis, the decreased measurement error of the more heritable voxels could result in the more significant association of genetic variants. In other words,

the enhancement of signal is a reflection of a higher signal to noise ratio. Also a higher test re-test reliability of the highly heritable voxels, reduce signal to noise ratio. **Third, we used the same data for the calculation of population-based heritability estimates and genetic testing, resulting in a possible inflation of the increase in signal due to non-independence [Kriegeskorte, et al., 2009]. However, when only the family-based heritability estimates were used to select the voxels for genetic associations (Figure 3 C,D) the analyses were independent. In these analyses, we still observed an increase in the signal – and the enhancement was actually even stronger for the putamen – arguing against inflation due to non-independence. However, for the hippocampus the best enhancement was achieved using the combined sample when restricting to less than 55% the most significant voxels. While this could be due to non-independence, this is contradicted by the fact that the population-only results (i.e., fully dependent) are in fact worse at this and lower percentages. An explanation other than non-independence could be that the combined sample provides more accurate heritability estimates and therefore results in a better enhancement.** Last, highly heritable voxels which are in close proximity of each other could share their genetic background. However finding a cluster of heritable voxels does not directly prove genetic correlation.

Strengths and limitations

Major strengths of this study are the large sample size of the population based study and unified imaging processing. Subjects from ERF and the Rotterdam Study subjects were scanned using the same 1.5T scanner, identical MRI protocols and images were processed with exactly the same software. The ASPS-Fam was scanned on a 3T scanner, but segmented using similar protocols and VBM processing was performed in the same way as ERF and the Rotterdam Study. Important to note is that softwares used for tissue segmentation are different, but both implement the same kNN algorithm [Vrooman, et al., 2007]. The ERF and the Rotterdam Study both are both from the

Netherlands, a genetically homogeneous country [Boomsma, et al., 2014]. The ASPS-Fam study is from Austria, Austrians likely have slightly different genetic architecture than the Dutch**. Maximum likelihood iterative optimization was used to estimation heritability. The iterations are prone to convergence failures when sample sizes are small. The percentage of voxels that did not converge was 9% in ASPS-Fam ($N_{participants}$ = 369) and 36% in ERF ($N_{participants}$ = 122). The methods used for population-based estimation of heritability always output an estimate.** It has been shown that not converging occurs frequently in small datasets in SOLAR producing conservative estimates [Blangero, et al., 2013; Koran, et al., 2014]. We further note that using only VBM to assess heritability of brain morphology is a limitation of the current study. Cortical thickness, surface area and other MRI measures, **including tensor-based (i.e. deformation) morphometry (TBM) [Brun, et al., 2009; Yoon, et al., 2011]** and shape analysis are all potentially interesting for future heritability and genetic studies. The differences between measures have been attributed both to biology [Voets, et al., 2008; Winkler, et al., 2010] and methodology [Blankstein, et al., 2009; Hutton, et al., 2009]. Most probably, these measures reflect a different genetic architecture [Winkler, et al., 2010] and should therefore be studied separately.

Future perspectives

Genetic association with several voxels within an anatomical structure is biologically relevant as it shows an important genetic contribution to a sub region of the structure. Apart from the biological relevance, this sub region of voxels could have clinical significance. For example, it was shown previously that subfields of the anatomically defined hippocampus contributed differently to schizophrenia [Kuhn, et al., 2012] and β-Amyloid load [Schroeder, et al., 2016]. If only highly heritability brain voxels are studied in future voxel-wise genome-wide association studies we do not expect statistical signals to be uniformly enhanced. However, for the tested genetic variant that was identified for putamen volume, we did find statistical enhancement. High heritability estimates capture a variety of sources that can affect power to detect associations, including lower signal to

noise ratios and higher genetic homogeneity (i.e. genetic correlation). Using these benefits to increase statistical signal is desirable, irrespective of the underlying cause. Ideally we envision selecting groups of voxels for genetic studies based on high heritability and measured high genetic correlation. Genetic correlation can be calculated for any of the commonly used MRI-measures, but it would still require genetic testing of sufficiently powered (large) studies. A promising future direction would be to enable the calculation of genetic correlations, genetic association (millions of voxels times millions of genetic variants) and meta-analyses of these associations. Programs which make the calculation of genetic correlation and genetic association computationally possible in sufficiently powered studies (i.e. meta-analyses) are essential to the field. Currently these programs tailored to large scale genetic studies are developed and genetic studies started **[Roshchupkin, et al., 2016b]**. The results of these studies will be able to prove to which extend clusters of heritable voxels have a common genetic architecture.

Conclusions

Heritability estimates can be reliably estimated using different methods and on different cohorts and combining heritability estimates from multiple studies leads to the construction of a reliable heritability map of grey matter. These maps can be used to prioritize highly heritable regions in future genetic imaging studies.

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Figure 1: Example of FDR-Significant voxels in both population-based (**A**) and family-based (**B**) estimates. Significant voxels cluster in subcortical structures, such as the caudate nucleus. All results can be interactive accessed (www.imagene.nl/heritability) and downloaded from the website.

Figure 2: A Barplot showing regional brain heritability. Structures that are in both the left as well as the right hemisphere were averaged for this figure. It can clearly be seen that the heritability from family-based studies is higher than heritability form the unrelated population (P<0.001). **B** Scatter plot of the average regional heritability of all brain structures. The correlation of the family-based and population-based estimates was high (Pearson's correlation coefficient = 0.73, $p = 2.6 \times 10^{-13}$). Data points per structure correspond to family and population heritability in table 2.

Figure 3: Enhancement of the association signal of variants with the most heritable voxels of the hippocampus and putamen. **A,B:** Average heritability (y-axis) of the voxels in hippocampus (A) and putamen (B) given a percentage of the most heritable voxels in that region (x-axis) in steps of 5%. **C,D:** The -log(p-value) increase comparing the p-value of association with subsets of the most heritable voxels and all voxels in the region. The –log(p-value) increase for association of hippocampal with rs77956314 (*HRK* gene) and putamen voxels with rs945270 (*KTN1* gene) is shown. Associations were corrected for age, sex, and the first three principal components.

Supplementary Figure 1: Example of the intraclass correlation (ICC) in 83 individuals scanned twice within several weeks. In general voxels have a high ICC. All results can be interactive accessed (www.imagene.nl/heritability) and downloaded from the website.