Correlation-based multivariate analysis of genetic influence on brain volume

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\textbf{A R T I C L E  I N F O}

Article history:
Received 11 August 2008
Received in revised form 11 November 2008
Accepted 14 November 2008

Keywords:
Canonical correlation analysis
Kernel methods
Multivariate genetic analysis
Multivariate sMRI analysis

\textbf{A B S T R A C T}

Considerable research effort has focused on achieving a better understanding of the genetic correlates of individual differences in volumetric and morphological brain measures. The importance of these efforts is underlined by evidence suggesting that brain changes in a number of neuropsychiatric disorders are at least partly genetic in origin. The currently used methods to study these relationships are mostly based on single-genotype univariate analysis techniques. These methods are limited as multiple genes are likely to interact with each other in their influences on brain structure and function. In this paper we present a feasibility study where we show that by using kernel correlation analysis, with a new genotypes representation, it is possible to analyse the relative associations of several genetic polymorphisms with brain structure. The implementation of the method is demonstrated on genetic and structural magnetic resonance imaging (MRI) data acquired from a group of 16 healthy subjects by showing the multivariate genetic influence on grey and white matter.

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Macroscopic features of brain morphology and volume are known to be highly heritable [14]. Given the observation of volumetric and structural brain changes in a number of neuropsychiatric conditions [8,10], considerable research effort has focused on elucidating these changes in vivo using structural magnetic resonance imaging (MRI). The importance of these efforts is underlined by evidence suggesting that brain changes in a number of disorders are at least partly genetic in origin, as suggested by family and twin studies [2,3].

Recently, MRI studies have begun to identify specific molecular genetic candidates for these brain changes. In a typical genetic MRI paradigm, participants are classified on the basis of DNA analysis according to a certain genotype (e.g. genotype groups AA, AB, BB; or A-allele carriers vs. non-carriers). Statistical analysis, such as a t-test or analysis of variance (ANOVA), of MRI data is then carried out to identify differences between the groups which are assumed to reflect the influence of the investigated genotype. These single-genre association studies have been useful in characterising the likely mechanisms of risk genes on brain structure and function and, therefore, the pathophysiology of neuropsychiatric conditions [7,11].

However, the analysis of single-genre effects is limited as complex phenotypes are likely influenced by multiple genetic polymorphisms. Also, there are likely to be effects of epistasis, i.e. gene–gene interactions, on brain and function. The complexity of statistical analysis of such interactions calls for the development of novel statistical techniques that can identify which combination of genotypes can best explain statistical variance in brain structure and function.

Machine learning/pattern recognition methods are increasingly being used to analyze fMRI data. The most commonly employed method, the Support Vector Machine (SVM), a supervised method, associates properties of the imaging data with simple specific categorical labels (e.g. –1, 1 indicating experimental conditions 1 and 2). The aim is then to find the hyperplane that gives optimal separation between data belonging to the two classes, using data from the whole brain in each subject, for examples see Mourao-Miranda et al. [12,13]. In some situations, the use of categorical labels may not be optimal and in an earlier study [6], we introduced a new unsupervised fMRI analysis method based on Kernel Canonical Correlation Analysis (KCCA) to overcome this problem. KCCA replaces the simple categorical labels used in SVM (+1/ –1) with a label vector for each stimulus containing details of the features of that stimulus, e.g. a simple image label of pleasant/unpleasant is replaced by a vector of image features.

In this paper we extend the unsupervised application of KCCA to the analysis of genotypic effects on brain structure. We show that by
using KCCA, with a new orthogonalised representation to express the genotype labeling, it is possible to examine the influences of the interactions of multiple genes on brain structure.

16 healthy participants (14 males) took part. All participants were right-handed, Caucasian, and psychiatrically, medically and neurologically healthy. Ages ranged from 18 to 36 years (mean = 23.69, S.D. = 4.33) and participants had spent an average (S.D.) of 16.81 (2.64) years in full-time education. Participants provided written, informed consent. The study had permission from the local research ethics committee.

DNA was obtained from either buccal swabs or whole blood. The cheek swab method typically provides excellent yield and quality of DNA; cheek swab samples were sent to the laboratory at the Social, Genetic, and Developmental Psychiatry Centre (SGDP) at the Institute of Psychiatry, where DNA was extracted from the samples using established procedures [5]. DNA was extracted from whole blood by isolation of the white cells followed by a modified phenol-chloroform procedure. After extraction the DNA was re-suspended in water.

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23 neurologically healthy. Ages ranged from 18 to 36 years (mean = 28.2, S.D. = 3.3) and participants had spent an average (S.D.) of 16.81 (2.64) years in full-time education. Participants provided written, informed consent. The study had permission from the local research ethics committee.

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The grey and white matter probability images produced by SPM5 are of much higher accuracy than in SPM2 and modulation step somewhat smoothes the images, the grey and white matter tissue segments were smooth with 8 mm FWHM Gaussian kernel, which approximates smoothness of 12 mm obtained using SPM2 software. The grey and white matter probability images were resliced with 2 x 2 x 2 voxel size, since higher resolution of 1 x 1 x 1 would considerably increase the dimensionality of the classification problem, distributing any pattern in the data over a much broader area in term of search space for the classifier.

CCA is a technique, proposed by Hotelling [9] for finding pairs of basis vectors that maximise the correlation of a set of paired variables. These pairs can be considered as two “views” of the same object. This technique is applicable in cases where each “view” contains, as a subspace, all “relevant” information plus some “irrelevant” information. CCA identifies a projection space containing the relevant subspaces in both views. This projection space is often referred to as the semantic space. In the following study we consider the SNPs of the genetic sequence and the segmented white or grey matter of structural MRI brain scans to be two “views” of the same object. CCA seeks a pair of linear transformations one for each of the paired variables such that when the variables are transformed the corresponding coordinates are maximally correlated. In this paper we use the kernel variant of CCA. Due to space limitation we refer the reader to [6] for exact details and derivations.

In the following section we elaborate on our experimental setup, analyses and results. Our data set consisted of 16 subjects. Genotyping of the sample yielded 94 SNPs in each subject. Each SNP is coded as 3 genotypes, i.e. a subject is for any given SNP examined aligned here an A-allele homozygote (AA), a heterozygote (AB), or a B-allele homozygote (BB). Each subject also completed a structural MRI scan of the brain as described above. In our analysis we masked the voxels in the structural MR images using grey and white matter masks thresholded at voxel intensity values of 0.2 for both grey and white tissue images. This value was derived by visual inspection of the individual images. The regularisation parameter is heuristically fixed to \( \tau = 0.03 \) for the present preliminary study. The rigorous optimisation of this regularisation parameter will be addressed in a future study.

In the multivariate analysis we are unable to use the conventional univariate label representation of the SNP genotype of 1 (or AA), 2 (or AB) and 3 (or BB) due to the fact that we do not know a-priori the direction of each of the SNPs vector, i.e. whether the genotype representation 1 < 2 < 3 or 1 > 2 > 3 is true for each genotype.

SNP, although the direction for each SNP across the subjects is the same. We overcome this issue by representing the SNP genotypes in a three-dimensional orthogonalised label such that $1 \perp 2 \land 1 \perp 3 \lor 2 \land 3$. In other words we use the following representation of the genotypes:

1 $\rightarrow [0, 0, 1]$
2 $\rightarrow [0, 1, 0]$
3 $\rightarrow [1, 0, 0]$

as our corresponding label representation of the 3 genotypes. This method therefore also allows for the identification of heterozygosity effects (e.g. AB $>$ AA $=$ BB). The analysis thus is of additive genetic effects.

Following the KCCA procedure we wish to analyse the effects of all SNPs on entire, masked, brain. The inherited structure of KCCA entails that we implicitly incorporate the effect of the voxels on each other. This would have also been true for the effect of the SNP’s on each other but our orthogonalised representation entails that each SNP’s mean weight value will be zero. In other words, what we examined was the relative influence of the three different genotypes within the SNPs on grey and white matter. The resulting output weights from the proposed technique represent, on the one hand, the changes in brain matter volume and on the other, the corresponding relative influence of the genotypes within the SNP’s on the change in brain matter volume.

We address the question of methods reliability by randomly splitting the samples into two equal groups and comparing the resulting analysis of the groups, i.e. we compute a correlation value between the two resulting normalised genetic and MRI weight maps. We repeat this procedure 100 times and give the average correlation values and standard deviation in Table 1. The high correlation value and low standard deviation represent an agreement for the different analyses which in turn constitute an argument for the reliability of the proposed method.

To avoid overinterpreting our results due to the small sample size, we confine ourselves to a plot in Fig. 1 representing each SNP’s largest contributing (absolute) genotype value. This allows us to visualise the overall influence each SNP has on the grey and white matter. We normalise the 94 SNP vector so that we are able to compare between the grey and white resulting weight vectors. The individual allele contribution for each of the SNP weightings is given in Fig. 2. While there is a difference between the genetic influence on white and grey matter it is not apparent. We separate the individual genotypes weights and plot a direct comparison in Fig. 3.

The current study presented a method for the analysis of complex gene-MRI associations. We aimed to explore whether KCCA could usefully be applied to genetic (SNPs in genes thought to be related to human brain volume) and neuroimaging (grey and white matter extracted from structural MRI) data to detect multivariate correlations between genetic and structural imaging data. The results of the study are shown in two contexts: (1) the impact of genetic variability at each SNP on structural variation in grey or white matter volume and (2) the individual weightings of voxels in grey and white matter in terms of how much their variability was correlated with SNP variability across all SNPs.

Fig. 1 shows the SNP “loading” profiles with voxelwise volume variation of grey and white matter, e.g. the relative correlations of different SNPs with voxelwise volumetric variation within grey and white matter. The subjects were split into two random groups of eight subjects and the correlation of the resulting analysis between the two groups was computed. This process was repeated 100 times and is reported with the standard deviation.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Genetics</th>
<th>MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey</td>
<td>0.8707 ± 0.0289</td>
<td>0.8891 ± 0.0810</td>
</tr>
<tr>
<td>White</td>
<td>0.8111 ± 0.0455</td>
<td>0.8920 ± 0.1015</td>
</tr>
</tbody>
</table>

The subjects were split into two random groups of eight subjects and the correlation of the resulting analysis between the two groups was computed. This process was repeated 100 times and is reported with the standard deviation.
white matter areas of the brain. We heuristically threshold at 0.16 so to give the top $\approx 10\%$ of SNP with the largest influence. The SNP’s with the largest apparent correlations with volumetric variation are shown in Table 2. Figs. 4 and 5 shows the voxel-wise loadings in grey and white matter volume of SNP correlations, e.g. how much these voxels are under the combined genetic influence of all the SNPs studied. The maps were displayed using AFNI.\textsuperscript{3} The colour bar indicates mapping from the KCCA weights to colours. To generate a colour bar that is symmetric around zero the values in the weight maps are rescaled in such a way that the absolute maximum is assign the value of $+1$ and the colour scale runs from $-1$ to $+1$. The absolute maximum was 12.74 for the gray matter weight and 25.73 for the white matter weight. Both weights were normalised $(\text{normalised } w = \frac{w}{\|w\|_2})$ and multiplied by 1000. For each map the threshold corresponded a 20\% of their respective maximum.

We should be very conservative in our interpretations of the results from a small, preliminary study. However, it appears (a) the

\textsuperscript{3} http://afni.nimh.nih.gov.
method enables us to place some ranking on the possible importance of SNPs in driving structural brain change and (b) that the brain areas under apparent SNP-related influence fall into a number of broad regions. The regions shown in red and blue are affected in opposite directions but we do not at present read these as increases and decreases in grey or white matter volume. Considering our voxel size and a relatively coarse smoothing kernel in the present analysis, the results should be applied as representing associations between genes and large-scale volumetric variations. For fine-grained volumetric variations a higher image resolution (i.e. $1 \times 1 \times 1$) with smaller smoothing kernel (e.g. 6 mm) should be applied.

Broadly speaking, the red areas include cerebellum, occipital cortex, anterior cingulate and lateral frontal regions. The blue regions include posterior cingulate, parietal cortex and some temporal regions. We refrain at present from placing firm interpretations on these results pending replication and extension to larger groups. With this caveat in mind, however, it is of interest to note that all except two (rs909525 and rs6609257) SNPs with a weight influence of more than 0.16 (see Table 2) are from the microcephalin (MCPH1) gene. MCPH1 is thought to be involved in the regulation of human brain size [4] and has been shown to be associated with cranial volume in a recent study [15]. However, it should be borne in mind that linkage disequilibrium amongst SNPs in the same gene is common; this potential problem should be addressed in future studies.

We conclude by arguing that KCCA with a linear kernel has potential to investigate the interactional effects of multiple genetic influences on brain structure. We hope that the possibilities raised by this method will be usefully exploited to clarify the nature of multiple gene influences on brain structure and function in large samples of healthy and diseased subjects.

**Table 2**

SNPs with a weight influence greater than 0.16 on grey or white matter.

<table>
<thead>
<tr>
<th>SNP (grey)</th>
<th>SNP weight (grey)</th>
<th>SNP (white)</th>
<th>SNP weight (white)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(45) rs17076812</td>
<td>0.1664</td>
<td>(19) rs909525</td>
<td>0.1713</td>
</tr>
<tr>
<td>(47) rs1129703</td>
<td>0.1664</td>
<td>(21) rs6609257</td>
<td>0.1635</td>
</tr>
<tr>
<td>(48) rs1129706</td>
<td>0.1664</td>
<td>(64) rs2515477</td>
<td>0.1601</td>
</tr>
<tr>
<td>(49) rs2034143</td>
<td>0.1664</td>
<td>(80) rs2442572</td>
<td>0.1665</td>
</tr>
<tr>
<td>(51) rs2440399</td>
<td>0.1608</td>
<td>(84) rs2959802</td>
<td>0.1676</td>
</tr>
<tr>
<td>(52) rs12674488</td>
<td>0.1652</td>
<td>(86) rs2959799</td>
<td>0.1681</td>
</tr>
<tr>
<td>(72) rs2515493</td>
<td>0.1717</td>
<td>(87) rs2013938</td>
<td>0.1634</td>
</tr>
<tr>
<td>(80) rs2442572</td>
<td>0.1745</td>
<td>(88) rs1057090</td>
<td>0.1676</td>
</tr>
<tr>
<td>(90) rs2911968</td>
<td>0.1676</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The SNP number in parentheses corresponds to the numbering of the SNPs in the order as they are presented.
Fig. 5. Weighted MRI of genetic influence on white matter volume. The colour bar indicates mapping from the KCCA weights to colours. The blue and red represent opposite change influence where blue is the negative values and red is the positive values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Acknowledgements

We are grateful for partial support from an EC FP6 grant, Project 037761, “SGENE”. David R. Hardoon is supported by the EPSRC (National Institute for Health Research) Personal Award. The views expressed in this publication are those of the authors and not necessarily those of the NHS, NIHR or Department of Health.

References