Machine learning as a statistical tool in schizophrenia research

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Abstract

Schizophrenia is a heterogeneous and multi-factored disease. Investigation of the disorder could profit from statistical methods which can address multiple putative factors and large, complex datasets. Machine learning is a branch of statistical analysis which has specialized in developing such methods. This dissertation contains four investigations of schizophrenia, each highlighting a different aspect of how machine learning can address topical questions in schizophrenia research.

The first study, "Potential genetic variants in schizophrenia: A Bayesian analysis," tested 36 candidate genetic loci to identify those which associated with increased risk of schizophrenia. Genetic effect sizes are small, requiring large samples to detect. Yet certain potentially interesting genetic variants are rare, making collecting such samples difficult. Early selection of genes worth further pursuit can save much wasted time and effort. Six loci were indicated.

The second study, "Morphological correlates to cognitive dysfunction in schizophrenia as studied with Bayesian regression," compared a set of brain morphological measures to identify those which best explained cognitive skill scores. Measures included volumes of cortical, subcortical, and cerebellar structure selected to reflect conflicting models of the morphological substrates of cognition and cognitive deficit in schizophrenia. It found that subcortical and cerebellar structures better explained cognitive skill than cortical structures.

The third study, "Investigating possible subtypes of schizophrenia patients and controls based on brain cortical thickness," searched for cortical regions which showed evidence of morphologically distinguishable subtypes. The clinical heterogeneity of schizophrenia suggests that many disease factors may lead to morphologically distinguishable subtypes in patients. The same method applied to a mixed sample of case and control subjects provided a non-parametric investigation of cortical thickness variation in the disease. Morphological subtypes were not found in the patients. One third of the cortex was found to have two distinguishable types when patients and healthy control subjects were examined together.

The fourth study, "Grey and white matter proportional relationships in the cerebellar vermis altered in schizophrenia," hypothesized that proportional relationships between grey and white matter tissue volumes in the vermis would be strong in healthy control subjects and weakened in patients, reflecting an optimum balance dictated by contrasting biological constraints and disturbed in the disease. This was found to be the case, suggesting an alternate model for vermis neuropathology in schizophrenia.

These studies show that machine learning can identify promising avenues for further exploration, discern among overlapping hypotheses, elucidate the structure of the data, and allow the formulation of novel hypotheses based on the structure of the data.

List of studies

I. H Hall, G Lawyer, A Sillén, EG Jönsson, I Agartz, L Terenius, S Arnborg. Potential genetic variants in schizophrenia: A Bayesian analysis. *The World Journal of Biological Psychiatry* 8(1):12–22, 2007.

II. G Lawyer, H Nyman, I Agartz, S Arnborg, EG Jönsson, G Sedvall, H Hall. Morphological correlates to cognitive dysfunction in schizophrenia as studied with Bayesian regression. *BMC Psychiatry* 6:31, 2006.

III. G Lawyer, R Nesvåg, K Varnäs, A Frigessi, I Agartz. Investigating possible subtypes of schizophrenia patients and controls based on brain cortical thickness. *Psychiatry Research: Neuroimaging* [in press].

IV. G Lawyer, R Nesvåg, K Varnäs, G Okugawa, I Agartz. Grey and white matter proportional relationships in the cerebellar vermis altered in schizophrenia. *In manuscript.*

Contents

List of abbreviations

BA	Brodmann area
BCS	Bayesian covariate selection
BDNF	Brain Derived Neurotrophic Factor
COMT	Catechol-O-methyl transferase
CPT	Continuous Performance Test
CSF	Cerebral-spinal fluid
DISC1	Disturbed in schizophrenia
DRD2	Dopamine D2 Receptor
DSM	Diagnostic and Statistical Manual of Mental Disorders
FDR	False Discovery Rate
fMRI	Functional Magnetic Resonance Imaging
GM	Grey matter
ICC	Intra-class correlation coefficient
LNS	Letter-Number Sequencing
MCMC	Markov Chain Monte Carlo
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
NPY	Neuropeptide Y
NRG1	Neuregulin
PCA	Principle component analysis
PCR	Polymerase chain reaction
RAVLT	Rey Auditory Verbal Learning Test
RELN	Reelin
ROI	Region of interest
SNP	Single-nucleotide polymorphism
SYN	Synapsin
TMTA/TMTB	Trail Making Test Form A/Form B
WAIS-R	Wechsler Adult Intelligence Scale–Revised.
WCST	Wisconsin Card Sorting Test
WM	White matter

1 Introduction

The thesis which guided this work was that machine learning techniques could provide insight into concepts and theories highly relevant to current understanding of schizophrenia. The use of alternative methodology was motivated by changes in the nature of contemporary medical research and by the complexity of the schizophrenia disorder. Machine learning provided a solid mathematical framework and robust tools for the analysis of the data.

Medical knowledge is becoming increasingly specialized. One hundred years ago it was possible for a person to know everything that was known about schizophrenia. Today's body of knowledge is too broad for a single researcher to be well versed in every relevant aspect. It is the rare researcher indeed who has specialist level knowledge in clinical issues, neurocognitive testing, molecular biology, functional neuroimaging, structural neuroimaging, and psychopharmacology. All of these fields have much to contribute to our understanding of schizophrenia.

Along with this increase of knowledge comes an increase in the amount of data collected from each subject. Many current research projects involve several specializations. Subjects may be characterized across each of these. Each domain may include numerous measures, and the measures within each domain may contain structural relationships. Neurocognitive testing can cover several aspects of cognition and scores may be correlated. Magnetic Resonance Imaging (MRI) of the human brain can produce images with 100,000 or more data-points and strong spatial dependencies.

The dual trends towards increased specialization and complex datasets generalize to many areas of current research. Schizophrenia research, however, has an additional compelling reason to adopt machine learning techniques. Traditionally, medical research has been hypothesis-driven. Despite 100 years of such research, and numerous strong results, some of the most fundamental questions regarding schizophrenia remain unanswered [2]. While diagnostic instruments such as structured interviews based on the Diagnostic and Statistical Manual of Mental Disorders (DSM) claim high inter-rater reliabilities, the disease is quite heterogeneous in its manifestation. The heterogeneous, multi-factoral nature of schizophrenia suggests that investigation of the disease may profit from statistical approachs which can identify patterns in the data.

This has lead contemporary researchers to form large cross-domain research groups, such as the Human Brain Informatics (HUBIN) project, established in 1998 at Karolinska Institutet, Sweden. HUBIN investigates behavioral, brain imaging, environmental, molecular genetic, and phenotypic data for representative schizophrenia patient populations and healthy control

subjects. HUBIN was formed to facilitate the simultaneous investigation of multiple hypothesis possibly covering several specialties. An explicit goal of the project was to use machine learning to explore the nature and causes of schizophrenia.

1.1 Schizophrenia

Schizophrenia is a multifaceted disease that evades easy characterization. The disease is not rare, with a lifetime risk commonly quoted as 1% [2, 61, 78]. This disease causes great harm, both in terms of individual suffering and cost to society. The importance of this disease has lead to a great deal of research effort into its nature and causes over the last 100 years. While much is now known about the disease, the knowledge is in many ways fragmentary. Disparate findings are common. Its etiology is not known. It is not even certain if the disease is neurodevelopmental or neurodegenerative, a debate which goes back to the time of Kraepelin (1919) [54].

Schizophrenia symptoms are commonly grouped along three dimensions: positive, negative, and disorganization [61]. The term cognitive impairment is also commonly used in place of disorganization. Positive symptoms include hallucinations, delusions, and thought disorders. Negative symptoms include reduced volition and affect, apathy, and alogia. Disorganizational symptoms include loosening of associations and loss of ability to maintain a trail of though. It is not clear, however, that the three dimension model best characterizes the different symptoms of the disease [20]. Nor is it clear that it adequately describes the patterns of symptom co-occurrence observed in patients [79, 80]. Symptomatic manifestation is highly heterogeneous.

Cognitive imparement is one of the main features of schizophrenia. This can take the form of reduced attention span, reduced executive ability, and difficulties with learning and memory [61]. While patients as a group tend to have lower scores than healthy controls on cognitive skill tests, it is fairer to characterize patients as having greater variance in their scores. In one study of cognitive deficits in schizophrenia, the two highest scores on one of the cognitive tests were attained by patients [50].

Brain morphological findings are inconsistent. There is general agreement that schizophrenia is associated with pervasive brain morphological deficits, yet the to-date most comprehensive review of MRI findings concluded that the exact nature of these deficits is not clear [78]. The only consistent finding in [78] was reductions of grey matter in the superior gyrus of the temporal lobe. Even this finding is not universal. A later review of voxel-based morphometry studies found superior temporal gyrus grey matter reductions in only 57% of included studies [42]. While a number of converging lines of re-

search strongly imply that white matter deficits characterize the disease [16], diffusion tensor imaging has yet to detect reproducibly consistent differences in white matter integrity in schizophrenia [47].

Nor is it known if observed morphological changes represent the disease itself, either as cause or consequence, or merely an increased risk of disease. Both enlarged ventricles and reduced brain volumes are also found in non-affected family members [61]. Some evidence suggests that degree of morphological abnormality is associated with outcome. An investigation of people with prodromal signs of schizophrenia found that subjects who developed schizophrenia had less grey matter in the right medial temporal, lateral temporal, and inferior frontal cortex, and bilaterally in the cingulate, than those who did not [68]. But the evidence is not conclusive. Longitudinal studies have found increased enlargement of the lateral ventricles among patients with a poor outcome compared to patients with a better outcome [39, 53] as well as the reverse [17]. It is also possible that some brain changes represent the effects of antipsychotic medication [74].

Schizophrenia can be conceived as a disease of connectivity. The disconnectivity has been characterized as reduced neuropil [77], leading to deficit in local connections between grey matter neurons. Disconnectivity has alternately been explored in terms of large, whole brain networks. These may take the form of oligodendroglia [40] and/or other white matter dysfunction [43], or dysfunction in hypothesized prefrontal-thalamic-cerebellar networks [3, 43]. Some researchers have concentrated on functional disconnectivity [26], or correlation of mental activity between cortical regions, without exploring the underlying mechanism. The disconnectivity hypothesis has been simulated using artificial neural networks, in which it was possible to reproduce schizophrenia-like behavior [41].

The disease has a strong genetic element. Estimates of the heritability of the disease from twin studies range from 83% to 87% [10]. Unlike conditions such as Huntington's disease, which arises from a single, known, genetic variant, schizophrenia seems to be attributable to combination effects from multiple genes [33, 36]. Research has produced a long list of candidate genes, with associations including neurotransmission (COMT, DRD) [36, 45], neuronal growth (BDNF, DISC1, NRG1) [44, 76], and mylenation [32, 40]. It may be that several different and partially distinct sets of genes each expose a different risk factor or factors [8]. Some suspect sex-linked genes associated with language and cerebral lateralization [14] underly the disease. Women are less likely to be affected, and tend to have better disease outcomes than men [61].

Several environmental factors also show association with increased risk of disease. Possible stressors range from birth complications [9] to oxidative

stress [90]. Other evidence associates increased risk of disease with maternal fever or infection [58]. This suggests that a stress-diathesis process underlies the etiology.

Yet the neurodevelopmental model has some weakness. Primary among these is the long latency of the disease. First symptoms do not generally appear until early adulthood [58]. Treatment may to modify the course of the illness, which can be interpreted to imply that the underlying pathology is degenerative [54, 58]. The morphology exhibits neurodegenerative traits such as ventricular enlargement and brain volume reductions [78]. This degeneration could arise due to genetically influenced molecular disruption of neural circuits [36]. The pathophysiology of many neurodegenerative disorders can be traced in part to genetic variations which code for abnormal and potentially toxic proteins [58]. We cannot yet be certain that schizophrenia does not follow this pattern.

1.2 Machine learning

Machine learning is the science of inducing patterns from data. Data is analyzed to identify likely models, or hypotheses, within a specified framework. It can determine which out of a set of a priori plausible models are well supported by the data. Data can alternately be analyzed to identify its structure. This can show if further investigation is warrented to link explanatory factors to observed structure. It also allows for investigations of changes in structure between two conditions.

The roots of machine learning can be traced back to mathematicians such as Thomas Bayes (circa 1702–1761) whose eponymous rule allows for strict mathematical reasoning with probabilities, and Karl Pearson (1857–1936) whose research on correlation and regression analysis was designed to find associations in large datasets. The field grew rapidly in importance with the advent of computers (1945–) as researchers explored the new possibilities offered by a machine which could perform logical reasoning and numerical calculation.

1.2.1 Model selection

Regression analysis is one of the most common methods for identifying relationships between explanatory and dependent variables [12]. Covariate (or variable) selection is the branch of statistics concerned with determining which factors should be included in a regression model. Such problems are well known in epidemiology, as well as many non-medical fields. For example, an investigation of the effects of fat and alcohol consumption on breast can-

cer risk should consider age, education, menopausal status, age at menarche, family history of breast cancer, history of benign breast disease, and body mass index. The investigator must also consider if these variables should be coded as continuous or categorical variables, and if categorical, appropriate cutoffs must be chosen [70]. Different choices of which factors to include and how to encode them lead to a number of different models.

Bayesian analysis can be used to select covariates which appear to have an influence on an outcome. The goal of a Bayesian analysis is to determine the probability of each model, or model component, given the data [28]. Approaching the problem from a Bayesian point of view offers a natural way of addressing concerns over how encoding of the data or including/deleting covariates to/from the model affects its validity [12, 71]. Models can be compared or combined based on their probability.

Model selection can, of course, be done using significance testing. Generally some form of stepwise regression is used, where variables are included or excluded in the final model based on the results of F– or t–tests. Significance testing, however, is problematic in these situations, as it is based on the false assumption that only two models are under consideration. Further, such a process ignores model uncertainty [71]. Different selection procedures can lead to different models. As all candidate variables should have some theoretical justification, each of these differing models is defensible. Yet they can lead to radically different interpretations. The advantage of a Bayesian approach is that one has empirical grounds on which to compare these interpretations [71].

1.2.2 Exploring structure

Machine learning also offers tools for describing the variance of the data. One can test for the presence of clusters, which can reveal patterns that might not otherwise be obvious. Observing the nature of the clusters found in the data allows researchers to formulate more realistic models. Cluster-based models of symptom patterns in schizophrenia provide better fit to observed patient data than factor-based models [79, 80].

Cluster analysis allows the researcher to test for the existence of distinct groups in the data without having to specify a priori what causes the groupings. This is useful when many options are a priori reasonable. The heterogeneous nature of schizophrenia, for example, suggests several factors which could be associated with altered brain morphology. Two immediate possibilities include symptom profiles [57, 73] and medication effects [74]. Interactions between these two factors are also possible. Gender, though once suspected to be a factor, now appears to be less likely to affect morphological

changes in schizophrenia [62]. The presence of distinct subgroups of patients based on brain morphology could explain some of the discrepant findings in the literature.

Observing the patterns of correlation in the data can suggest novel linkages between elements. The study of correlations in fMRI time series inspired the concept of functional (dis)connectivity in schizophrenia [26], which has become a central paradigm of fMRI investigations into many areas. Correlations between regional brain volumes have been used to support the concept of dysfunctional fronto-temporal inter connections in schizophrenia [59, 60].

Clustering and correlation are linked. One of the primary clustering algorithms, k-means, has been shown to correspond with one of the primary algorithms for identifying sources of variance, principle component analysis [19]. Karl Pearson's influence on this science is witnessed by the common use of his test for correlation. The underlying concept of PCA was laid out by Pearson in 1901 [69].

It could be argued if correlation is machine learning or standard significance testing. Correlation is one of the most fundamental tests of association between two variables. It is commonly used in single-hypothesis investigations. Robert Fisher, however, was of the opinion that correlation was for exploratory analysis, not establishing hypothesis [25].

1.2.3 Other uses of machine learning

The examples of machine learning just given would fall into the category of unsupervised learning. Unsupervised learning, as just discussed, infers a model or models from the data. The other main branch of machine learning is supervised learning. Supervised learning creates or optimizes a model based on known input/output pairs. Often the goal is for the machine to learn to correctly classify inputs.

Researchers have recently begun to use supervised learning in place of significance tests. A classifier is trained to distinguish between two conditions, say case and control subjects, based on certain measures. If the classifier can then correctly classify new subjects with better than random chance, it is presumed that a difference exists between the two conditions [31]. The elegance of the approach is that the actual difference between the two groups does not need to be specified by the researcher. Specifying the exact difference can be very difficult when analyzing high-dimensional and highly structured data, such as an MR image. A telling example comes from an study of vision. A classifier was trained to distinguish the orientation of a visual stimulus based on the fMRI signal from the voxels of the visual cortex. It attained an accuracy of 80%, showing that real difference existed between

the different conditions. The difference, however, was invisible to voxel-wise t-tests statistics [37].

2 Aims

The overriding aim of this dissertation was to demonstrate that a machine learning based statistical approach could provide insight into some of the complexities of schizophrenia. The four included studies were selected to highlight different aspects of how machine learning could contribute to our understanding of the disease. The first study identified promising directions for future research. The second study searched across several biological models to identify the most likely explanation for the data. The third study tested for the presence of distinct subgroups in the data without a priori reference to factors which might explain such groups. The fourth study explored structural relationships in the data, and tested if these relationships were altered in schizophrenia.

The studies themselves aimed to investigate specific aspects of the disease. Each of the four included studies addressed a clinically relevant issue in schizophrenia research. The specific aims of each study, along with a brief motivation, are elaborated below.

2.1 Specific aims

The aim of the first study was to identify single-nucleotide polymorphisms (SNPs) in coding regions of candidate genes which showed an association with increased risk of disease. Schizophrenia has a strong hereditary component [10]. While many promising candidate genes have been suggested (e.g. [8, 36, 40, 44, 45, 76]), the actual genes involved are not known. Genetic investigations face several hurdles. Effect sizes tend to be small. Large samples are necessary to demonstrate significant effect. The rarity of some of the potentially relevant genotypes makes gathering large samples difficult. Identify SNPs of interest early in the research project can spare much wasted effort.

The aim of the second study was to identify relationships between brain structure volumes and cognitive performance, and differences in such relationships in patients with schizophrenia. Several models attempt to explain relationships between cognitive deficits and brain morphological changes observed in schizophrenia. These include less gray matter in the brain cerebral cortex [78], alterations in hypothesized neural circuitry involving the basal ganglia [78], and alteration in cerebellar structures and related neural circuitry [3]. Brain structures suggested by each of these theories were included in this study. Generally grey matter volumes of each structure were used, with the exceptions of the ventricles (cerebral-spinal fluid) and corpus callosum (a white matter structure).

The aim of the third study was to search the cerebral cortex for locations where subject cortical thickness might exhibit subtypes. As discussed in the introduction (sec 1.1), schizophrenia is a highly heterogeneous disease. This suggests the possibility that patients could subtype based on cortical thickness measures. Symptom differences [57, 73] and medication differences [74]. are only two possible dimensions which could associate with morphologically-based patient subtypes. Along with this intrinsic interest, finding evidence for subtypes could help explain the many discrepancies in studies of cortical grey mater differences between patients and healthy controls. By further including control subjects, such analysis provided a non-parametric investigation of disease-related cortical thickness variation. This provided valuable contrast with existing studies of cortical thickness variation in schizophrenia ([49, 63, 64, 88]) which all make strong assumptions regarding the data.

The aim of the fourth study was to test for proportional relationships between anatomical and tissue class divisions of the cerebral vermis in healthy control subjects, and to test if smaller vermis volumes reported in schizophrenia reflected disruption of these proportional relationships. It has been theorized that the vermis is involved in coordinating cognitive processes [75]. Associations between the vermis and some elements of cognition were found in study II of this dissertation [50]. Irregularities in hypothesized cerebellar-thalamic-cortical circuitry may underlie some of the symptoms of schizophrenia [3]. The vermis may be smaller in patients with schizophrenia than in healthy controls [66], though this finding is not universal [81]. An alternate possibility rests on the assumption that functional and biological constraints dictate optimal proportional relationships between morphological features. This possibility has been extensively explored in the mammalian isocortex [11, 46, 65] and specifically with reference to human intelligence [51], but not previously in the cerebellum. If the base assumption is true, then disruption of these scaling relationships could better describe vermian abnormalities in schizophrenia.

3 Materials and methods

3.1 Subjects

The subject material for this work was gathered as part of the Human Brain Informatics (HUBIN) project [5, 34], at Karolinska Institutet, Stockholm, Sweden, between 1999 and 2003. The full subject set included approximately 220 unrelated individuals evenly divided between patients with schizophrenia and healthy controls. Patients were recruited from outpatient psychiatric clinics in the Stockholm region. They were individuals with a stable, chronic diagnosis. All fulfilled DSM–III–R or DSM–IV criteria for a diagnosis of schizophrenia or schizoaffective disorder. Control subjects were drawn from a register of the general population or recruited from hospital staff. These individuals did not meet diagnostic criteria for any psychiatric disorder according to DSM–IV, and had no psychotic disorder among first-degree relatives.

Care was taken to match both age and gender across groups. Diagnosis or lack thereof was determined by structured clinical interviews conducted by a trained psychiatrist and, in the case of the patients, by review of hospital case notes [21, 84]. All subjects were healthy according to a clinical interview, physical examination, and biochemical screening. Exclusion criteria included a history of head trauma with loss of consciousness for more than five minutes, or somatic disorders affecting brain function.

After receiving a complete description of the study, all subjects gave written informed consent to participate. The study was approved by the Research Ethics Committee at Karolinska Institutet and the Swedish Data Inspection Board ("Datainspektionen").

3.2 Brain measures

Both T1– and T2–weighted Magnetic Resonance images (MRI) were acquired from each subject, under the following parameters. T1: 1.5 mm coronal slices, no gap, flip angle=35 degrees, TR=24 msec, TE=6.0 msec, number of excitations=2, field of view=24 cm, acquisition matrix=256x192. T2: 2.0 mm coronal slices, no gap, TR=6000 msec, TE=84 msec, number of excitations=2, field of view=24 cm, acquisition matrix=256x192. Scans were acquired using a 1.5 Tesla GE Signa (GE, Milwaukee, Wis, USA) system at the Magnetic Resonance Research Center, Karolinska Hospital, Stockholm, Sweden. The same instrument was used for all scan acquisitions. All scans were inspected by a neuroradiologist and found to be free of pathological defects.

3.2.1 Image analysis I

Regional and subcortical brain structure volumes used in studies II and IV were ascertained using the BRAINS[1] software package [4], following published laboratory manuals. An affine transformation was used to align the MR images to Talairach space [82]. Talairach boxes were assigned to specific regions corresponding to the frontal, occipital, parietal, and temporal lobes, and to the subcortical region. The tissue composition of each voxel was ascertained using multi-spectral discriminant analysis [35]. Artificial neural networks were used to automatically trace the intracranial volume and some subcortical structures [56]. Tracings were manually corrected. A number of regions (corpus callosum, caudate, putamen, hippocampus, cerebellum, the posterior superior, posterior inferior, and anterior vermis, and cerebellar tonsil) were manually delineated.

The quantitative analysis was performed blinded with regard to the two diagnostic categories by two specialists in psychiatry with at least one year of postdoctoral training. Test-retest reliability of the automatic segmentation has been ascertained [35], as has operator and inter-operator reliability of the manual tracing [1, 67]. The intra-class correlation coefficient (ICC) from ten scans investigated for intracranial volume, total grey matter, total white matter, and total cerebral-spinal fluid (CSF) ranged between 0.996 and 0.998. ICCs for test-retest of 11 different subjects rescanned after one month segmented by one operator were greater than 0.98 for total grey matter, white matter, and CSF classes. Manually delineated vermis regions displayed ICCs greater than 0.95 [66].

Study II used grey matter tissue volumes for all structures except the following three. For the ventricles, the volume of CSF in the central and lateral cavities was measured. For the corpus callosum, the white matter tissue volume contained in the three (1 mm thick) mid-sagittal slices of the structure was used. The total intracranial volume included all tissues inside the cranium. All measures were the sum of measurements taken from both hemispheres. This eliminated issues regarding the placement of the separating plane when considering small midline structures. Study IV used grey and white matter tissue volumes from the vermis.

3.2.2 Image analysis II

Cerebral cortex thickness measures used in study III were calculated from the T1–weighted images using the FreeSurfer[2] software version 1.2 [15, 22, 23].

[1] http://www.psychiatry.uiowa.edu/mhcrc/IPLpages/BRAINS.htm
[2] http://surfer.nmr.mgh.harvard.edu/

This method of estimating cortical thickness uses both intensity and continuity information from the entire MRI volume in segmentation and deformation procedures to construct representations of the gray/white matter boundary and pial surface. FreeSurfer calculates the distance between the grey/white matter boundary and the pial surface at numerous points (vertices) across the cortical mantle [22]. The maps produced are not restricted to voxel resolution of the original images and are thus capable of detecting sub millimeter differences between groups. The method has been validated by both histological [72] and manual measurements [49]. FreeSurfer version 3.0 was used to automatically parcellate the cortical mantle into discrete structures based on major gyral folding patterns [18, 24]. Cortical grey matter volumes within these structures was automatically measured. The parcellation and volumetric measures used the cortical surfaces generated with FreeSurfer version 1.2 as just described.

Topological defects in the automatically determined gray/white matter boundary were manually fixed by laboratory technicians who were instructed and supervised by senior researchers at the Institute of Psychology, UiO, Oslo, Norway. All analyses were performed blinded to subject identity.

3.3 Neurocognitive measures

Neuropsychological testing consisted of a standardized battery of neurocognitive tests covering 6 functional domains. All tests were administrated in a standardized order by a trained psychologist. Care was taken not to induce undue stress or fatigue in the patients. Verbal learning was assessed using the Rey Auditory Verbal Learning Test (RAVLT) series [52]. Vigilance was measured using a 150-item version of the Continuous Performance Test Identical pairs (CPT) [13]. Visuo-motor speed was measured by the Trail Making Test Form A and Form B (TMTA/TMTB) [52]. Working memory was assessed with the Letter-Number Sequencing (LNS) subtest from the WAIS-III [87]. Vocabulary, a rough premorbid functional indicator, was measured using the Vocabulary subtest of the WAIS-R [86]. Executive function was measured using the 64-card version of the Wisconsin Card Sorting Test (WCST) [38].

3.4 Genetic measures

Thirty candidate genes with putative association with schizophrenia were selected for analysis. Genes were selected if either they or their coded protein were associated with schizophrenia in previous independent research efforts. Thirty-six SNPs were selected on these 30 genes which met the following

criteria: located in the coding region of the gene, having a minor allele frequency of at least 5% in the general population, and reported by two groups as being associated with schizophrenia.

Genetic data was gathered by pyrosequencing of venous blood [27, 44]. Pyrosequencing used published polymerase chain reaction (PCR) primer sequences when they were available, otherwise new PCR primers were designed. Pyrosequencing followed the protocol specified by the manufacturer of the equipment (Pyrosequencing AB, Uppsala, Sweden), and was performed at Karolinska Institutet.

3.5 Bayesian covariate selection

Study I identified promising SNPs by evaluating the likely range of each regression coefficient in logit models expressing risk of schizophrenia as a function of SNP allele genotype. The true value of these coefficients cannot be known without infinite data, but a Bayesian analysis can fully describe the probability distribution capturing the uncertainty regarding their true value [28]. This allowed the calculation of an interval which was 95% likely to contain the true value, the 95% *credible interval*. If this interval excluded zero, then the associated variable was selected. The motivation for this criteria is that if a variable does have a true effect, that effect should be in one direction. When the data does not provide enough evidence to determine the direction of effect, then the effect is assumed to be non-existent or too small to meaningfully contribute to the model [28].

The data was not sufficient to allow testing of all SNPs in one model. They were instead tested in groups of three, rotating over different choices of which three were included in the model. The credible interval was determined via Markov Chain Monte Carlo (MCMC) carried out using the BUGS[3] engine [30].

Study II identified morphological measures associated with cognitive skill by comparing the probability of different linear regression models, given the data. The dependent variable was cognitive performance in one of 6 domains, many of which had multivariate measures. Independent variables were volumetric measures of 16 cortical and subcortical brain structures, as well as age, gender, and diagnosis.

Bayes' theorem shows that the probability of a model given the data is proportional to the probability of the data given the model scaled by the probability of the model. Covariate selection can be made by searching the space of all possible models which contain the factors of a priori interest. A

[3] http://www.mrc-bsu.cam.ac.uk/bugs/

covariate which appears in many highly probable models is likely to have an effect. The model space here consisted of all possible linear combinations of the independent variables, and interactions between the morphological measures and diagnosis. The model space was searched using a simulated annealing algorithm using a Bayesian decision-theoretic motivated heuristic to drive convergence to the most likely model [7].

3.6 Cluster analysis

Cluster analysis was used in study III to explore the structure of the cortical thickness measures. The primary goal of the analysis was to determine the number of clusters which optimally described the between-subject variation in the cortical thickness measurements. This was done by comparing the penalized error associated with different numbers of clusters.The optimal number of clusters was computed independently at numerous densely sampled cortical locations.

For each given number of clusters, the data was partitioned using the Llyod's k-means algorithm [55]. In k-means, the investigator decides a priori on the number of clusters k to be identified. Each datum is assigned to the nearest cluster center. These assignments are used to re-estimate the true cluster centers, data are reassigned, and centers re-estimated. The processes iterates until convergence, at which point the between-cluster variance has been maximized and within-cluster variance has been minimized. While the classification does assume a number of clusters, it does not assume that the clusters represent known distinctions such as patient/control.

The optimal number of clusters was determined by finding the number of clusters which minimizes the sum of within-cluster variances. As using more clusters automatically lowers this sum (within-cluster variance is zero when each datum is its own cluster), a penalty was added based on the number of clusters used. The penalty used was a derivative of the Bayesian Information Criteria, as it is asymptotically consistent [48].

The clustering procedure was carried out in a vertex-based morphometry context. Vertex-based morphometry applies a statistical test at each vertex of brain cortical maps. The goal of such an investigation is to describe cortical regions based on the results of the test.

Study III first tested a group of 96 patients to identify cortical regions which contained distinct subtypes based on cortical thickness. It was suspected a priori that subtypes, if indicated, would associate with different symptom profiles. Subtypes unique to the disease were not indicated by the analysis. The cluster analysis was repeated on a mixed sample containing the same 96 patients and 106 healthy controls. This provided a non-parametric

investigation of case/control differences in cortical thickness. Associations between clusters in the data and diagnosis were tested by contingency tables. This was followed up by significance testing for group differences in cortical grey matter volumes for those cortical structures which the cluster analysis found to be bimodal.

3.7 Correlation

Study IV tested the strength of scale relationships between tissue class volumes in the cerebellar vermis. Correlations between grey and white matter tissue volumes were tested both within and between three anatomically defined regions, the the posterior superior, posterior inferior, and anterior vermis. Strong correlations were interpreted as evidence of structural relationships. The study further tested if the cross-correlation matrix differed between patients and controls using the Box-M test.

The study used Benjamini and Hochbergs False Discovery Rate (FDR) [6] to control for multiple comparisons. FDR provides weak control against type I error, in that the expected proportion of false positives is kept below a user-defined threshold. It is useful in situations where the main hypothesis is established by a large number of supporting hypothesis, and where conclusions regarding the main hypothesis would not be invalidated if some of the reportedly true supporting hypothesis were false. FDR has proved to be a very popular approach to multiple comparison issues in a number of mass hypothesis testing fields, including genetics [83] and neuroimaging [29].

4 Summary of studies

Study I

H Hall, G Lawyer, A Sillén, EG Jönsson, I Agartz, L Terenius, S Arnborg. Potential genetic variants in schizophrenia: A Bayesian analysis. *The World Journal of Biological Psychiatry* 8(1):12–22, 2007.

Objective. Identify, from a set of 36 candidate single nucleotide polymorphisms (SNPs), those which were associated with an increased risk of schizophrenia.
Methods. Subjects included 103 patients with schizophrenia and 89 healthy control subjects. Thirty-six candidate SNPs, covering thirty genes, were selected which met the following three criteria: located in the coding part of the gene, reported to be associated with schizophrenia by at least two research groups, and with a minor allele frequency of at least 5% in the general population. Polymerase chain reaction primers applied to venous blood were used to determine subject genotypes. Logit linear models were used to represent the relationship between genotype and risk of disease. The credible intervals of the regression coefficients were determined using Markov Chain Monte Carlo; alleles whose regression coefficients were 95% likely to be non-zero were indicated. For contrast, standard significance testing in the form of Fisher's exact test for allele comparisons and a Chi-square test for genotype testing was applied to each SNP indicated by the above analysis.
Results. The confidence intervals indicated that the following genes showed association with increased risk of schizophrenia: BDNF, DRD2, NPY, NRG1, RELN, and SYN. The significance testing returned the following p-values, for allele and genotype comparisons respectively: BDNF p=0.26, p=0.20; DRD2 p=0.13, p=0.37; NPY p=0.43, p=0.78; NRG1 p=0.27, p=0.69; RELN p=0.22, p=0.21; SYN3 p=0.50, p=0.50.
Conclusion. A Bayesian approach was able to identify genes possibly involved in the etiology of schizophrenia, whereas a significance-testing based approach did not have sufficient power to indicate these genes.

Study II

G Lawyer, H Nyman, I Agartz, S Arnborg, EG Jönsson, G Sedvall, H Hall. Morphological correlates to cognitive dysfunction in schizophrenia as studied with Bayesian regression. *BMC Psychiatry* 6:31, 2006

Objective. Identify brain structures whose volumes associated with cognitive performance, and if such associations differed between controls and patients with schizophrenia.
Methods. Seventy-one patients with schizophrenia and 65 healthy control subjects were characterized by neuropsychological tests covering six functional domains. Measures of sixteen brain morphological structures were taken using semi-automatic and fully manual tracing of MRI images, with the full set of measures completed on thirty of the patients and twenty controls. Group differences were calculated. A Bayesian decision-theoretic method identified those morphological features which best explained neuropsychological test scores in the context of a multivariate response linear model with interactions.
Results. Patients performed significantly worse on all neuropsychological tests except executive function. The most prominent morphological observations were enlarged ventricles, reduced posterior superior vermis gray matter (GM) volumes, and increased putamen GM volumes in the patients. The corpus callosum was associated with verbal learning. Putamen GM volumes were associated with verbal learning, vigilance, and, to a lesser extent executive function, while caudate GM volumes were associated with working memory. Cerebellar vermis GM volumes were associated with vigilance, executive function, and, less strongly, visuo-motor speed. Those neuropsychological tests which were strongly associated to ventricular volume (visuo-motor speed, vocabulary, and executive function) showed only weak association to diagnosis, possibly because ventricular volume acted as a proxy for diagnosis. Diagnosis was strongly associated with the other neuropsychological tests, implying that the morphological associations for these tasks reflected morphological effects and not merely group volumetric differences. Interaction effects were rarely found, indicating that volumetric relationships to neuropsychological performance were similar for both patients and controls.
Conclusion. Subcortical and cerebellar structure volumes associated with cognitive skill while cortical volumes did not. The finding that a morphological indicator of diagnosis (ventricular volume) provided more explanatory power than diagnosis itself for visuo-motor speed, vocabulary, and executive function suggests that volumetric abnormalities in the disease are more important for cognition than non-morphological features.

Study III

G Lawyer, R Nesvåg, K Varnäs, A Frigessi, I Agartz. Investigating possible subtypes of schizophrenia patients and controls based on brain cortical thickness. *Psychiatry Research: Neuroimaging* [in press].

Objective. The clinical heterogeneity of schizophrenia suggests the possibility of patient subtypes based on localized variation in brain cortical thickness. Similarly, searching for cortical locations which subtype based on thickness in a mixed sample of patients and controls provides a non-parametric investigation of the cortical deficiencies known to exist in the disease.
Methods. Cortical thickness maps, generated from MR images of 96 patients with schizophrenia and 106 controls, were co-registered and corrected for age-related thinning. At multiple map locations, the number of subtypes best explaining cortical thickness in the patients, the controls, and both combined, was measured as the number of clusters with the lowest penalized error criteria score. Clusters were determined using Lloyd's k-means. Relationships between subject cluster membership and possible explanatory factors were measured using contingency tables. Grey matter volumes of bimodal regions in the combined subject group were measured, and the significance of group differences was determined using a Student's t test.
Results. Both patients and controls, considered independently, were predominantly homogeneous in cortical thickness. The few bimodal regions were similar in both groups. The combined subjects' cortical thickness was bimodal over 34% of the cortical mantle and otherwise unimodal. Further probing of these bimodal regions showed that subjects tending to belong to thinner modes were significantly more likely to be patients, and grey matter volumes of most bimodal regions were significantly smaller in patients.
Conclusion. The study found no subtypes specific to patients. It further found that the patients had distinctively thinner cortex than controls in large areas of the frontal and temporal lobes, and some regions of the parietal lobe.

Study IV

G Lawyer, R Nesvåg, K Varnäs, G Okugawa, I Agartz. Grey and white matter proportional relationships in the cerebellar vermis altered in schizophrenia. *In manuscript.*

Objective. Scaling relationships between morphological features have been shown in the mammalian isocortex. This study sought to establish scale relationships between grey and white matter tissue volumes in the cerebral vermis, and if the tissue reductions observed in schizophrenia preserved or disrupted these relationships.

Methods. Fifty-two chronic, stable, medicated patients (33 men, 19 women) with established diagnosis of schizophrenia (n=43) or schizoaffective disorder (n=9) according to DSM-III and DSM-IV criteria, recruited from outpatient clinics in the Stockholm region of Sweden, were compared with 55 age and gender matched healthy individuals (37 men, 18 women). The cerebellar vermis was divided into three regions, the anterior superior (lobules I–V), posterior superior (lobules VI–VII) and posterior inferior (lobules VIII–X). Regions were determined by manual tracing of structural MR images. Grey and white matter tissue volumes were measured for each region. Cross-correlations of the volumes were computed separately for patients and for controls, as were ratios of grey to white matter volumes within and across the three regions. Difference between the control and patient correlation matrices was measured using the Box M test. The individual correlations in the matrices were compared. Differences in the mean and variance of the ratios were assessed using t- and F-tests.

Results. The two correlation matrices were different (p=0.005). Fourteen of fifteen measured correlations were significant in the controls, while eight of fifteen were significant in the patients. Patients had significantly larger variance in all but one of the nine tissue class ratios. The means of the grey to white matter ratios were significantly higher in patients for five of the ratios.

Conclusion. Grey and white matter volumes within and across regions were strongly inter-related in healthy controls. These relationships were weakened in schizophrenia.

5 Results and comments

The studies included in this dissertation were selected to highlight different aspects of how machine learning can contribute to our knowledge of schizophrenia. The unifying focus of these studies was the use of alternative approaches, not biological findings. Each study's contribution, as well as possible improvements and follow-ups, is therefore addressed individually.

5.1 Study I

Study I was able to identify 6 SNPs from a field of 36 candidates which showed association with increased risk of schizophrenia using Bayesian methods. The six SNPs indicated lay on the following genes: BDNF, DRD2, NPY, NRG1, RELN, and SYN. Standard significance testing of these 6 SNPs did not show an association to increased risk in the same data.

Brain-derived neurotrophic factor (BDNF) regulates survival, differentiation, morphology, and synaptic remodeling of neurons. Analysis of a superset of the subjects from study I did not find associations between BDNF SNPs and diagnosis, but a meta-analysis of over 6000 subjects did find some associations between the BDNF gene and risk of disease [44]. BDNF has been shown to have a weak association with cortical thickness variations in patients with schizophrenia, though at a different SNP than that tested in Study I [85].

Many antipsychotic medications target the dopamine system, strongly suggesting that the dopamine D2 Receptor Gene (DRD2) is involved in the pathophysiology of schizophrenia. The analysis of a superset of the subjects from study I found a strongly significant ($p=0.002$) association between the DRD2 SNP indicated in study I and risk of schizophrenia [45]. The finding was supported by a meta-analysis of more than 9000 subjects.

Neuregulin (NRG1) is also believed to be involved in susceptibility to schizophrenia. The gene codes a number of proteins involved in neurotransmitters as well as neuronal migration and development [76]. A preliminary analysis of a superset of the subjects from study I showed a significant relationship between NRG1 and risk of schizophrenia (unpublished results).

Schizophrenia is thought to involve multiple genes, and/or result from a combination of genetic and extra-genetic causes. If true, the effect of any one SNP on odds ratios would tend to be quite small. Detection would require large samples to establish statistical significance. This is indeed the case. Individual studies have primarily negative findings, while meta-analysis of thousands of individuals are occasionally able to find associations [45]. Very large samples, however, carry a risk that inconsequential differences in odds

ratios are declared significant. Inference of biological causation must then be very cautious and treated with some skepticism

Study I treated genetic variation across SNPs as independent. Strictly speaking, the assumption of independence might not have been warranted. Certain SNP alleles are known to inherit together, in what are known as haplotypes [91]. It is unlikely that the various SNPs tested in the study formed haplotypes as they lay, for the most part, on different genes. This, however, was never tested nor discussed in the manuscript.

5.2 Study II

Study II identified brain structures whose volumes were predictive of cognitive skills. Verbal learning, as measured by the RAVL tests, was strongly associated with corpus callosum white matter volume and putamen grey matter volume. Vigilance, as measured by the CPT test, was strongly associated with putamen and vermis grey matter volumes. Visuo-motor speed, as measured by the TMT tests was weakly associated with vermis grey matter volumes. Working memory, as measured by the LNS test, was strongly associated with caudate grey matter volume Vocabulary, as measured by the WAIS-R vocabulary subtest, was weakly associated with vermis grey matter volumes. Executive ability, as measured by the WCST test, was strongly associated with the temporal lobe and the vermis. Interactions between morphological measures and diagnosis were rare, and never strongly predictive of cognitive scores.

In general, subcortical and cerebellar structure volumes proved more predictive of cognitive skill than cortical volumes. This provides evidence in favor of hypotheses claiming that the cognitive disruption characteristic of schizophrenia can be explained by alterations in these non-cortical structures. The lack of interaction with diagnosis suggests that the volumetric/cognitive relationships are not altered in the patients. This is especially intriguing, as both the volumes and the cognitive test scores differed between the two subject groups. One should not speculate too wildly, however, as putamen and caudate volumes are altered by many typical antipsychotic medications [74].

One potential weakness mentioned in the discussion of this study was that the measures of cortical grey matter were gross. It is not certain, however, that finer-scale measures would have proved advantageous. Several other investigations have observed relationships between global grey matter and cognitive performance, but not regional measures of grey matter [89]. Nonetheless, we have since tested for relationships between each of the cognitive domains and localized cortical thickness with a 1 mm sampling interval. Results from this analysis were primarily negative, with the exception of lim-

ited associations for the WAIS-R and WCST. This suggests that a finer-scale demarcation of the cortex in study II would have only slightly changed the results. Given that the further analysis had generally weak or negative findings, it is unlikely that the general conclusion of study II would have changed had finer cortical structure definitions been used.

A weakness not addressed in the study was if cortical volumes were the most appropriate measure. It has been suggested that correlation between cortical grey matter measures is more predictive of intelligence than the raw measures themselves [89]. A study of brain development in healthy adolescents found an increasing correlation with age between the left Brodmann Area (BA) 44 and its counterparts in the language network and frontal lobe circuits [51]. BA 44 is believed to be involved in language and speech processing. The same study found that subjects with higher scores on standardized IQ test had stronger correlations between BA 44 and the ventro and dorsolateral prefrontal cortex, the lateral parietal lobes, and the right anterior cingulate than those with lower scores. A study spanning two publications found that schizophrenia patients had abnormal patterns of correlation between cortical grey matter volumes [59, 60].

This suggest that had cortical grey matter measures in study II been replaced with correlational between these measures, the cortex may have been more predictive of cognitive skill. Such speculation, however, leaves open the question of what caused the correlation between grey matter structures. An idea expressed in all of the just cited works [51, 59, 60, 89] is that the decreased correlation reflects disturbances in connectivity between the regions. The corpus callosum is a major interhemispheric pathway, and the putamen, caudate, and vermis could very well mediate inter-regional connectivity by either directly or indirectly coordinating cortical activity.

5.3 Study III

This study searched the cortex to identify locations which contained distinct subtypes of cortical thickness. It was found that small regions of the cortex did exhibit subtypes in the patients. Findings were in the anterior temporal cortex, the superior portion of the precentral gyrus, and the anterior orbito-frontal cortex. These same regions, however, also exhibited subtypes in healthy control subjects. The regions were slightly larger in the controls, implying that the patients were the more homogeneous group. This did not give us confidence that relationships between subtypes and disease-related factors would have true biological meaning.

When the subject sample included both patients and controls it was found that 35% of the cortex was bimodal. When bimodal regions were considered

as a whole, diagnosis was significantly associated with membership in the thinner cluster. Follow-up analysis tested for volumetric differences in 20 anatomically defined structures [18] which corresponded to bimodal regions. Significant group differences in cortical grey matter volumes were found in 70% of the structures in the left hemisphere and 80% of the structures in the right.

The finding of morphological subtypes in limited cortical regions in both healthy controls and patients suggests that these regions may not fit distributional assumptions commonly made in group comparisons. There is an increased risk for false negatives in these locations. The regions were not large enough, however, to offer much explanation for the many discrepant findings in studies of schizophrenia. It is more likely that discrepant findings arise due to differences in methods, ROI definitions, and subject samples [42, 78].

An issue with cluster-based analysis is that factors underlying the clustering are not always obvious. In the case of the separate subject groups, no obvious candidate presented itself as the subtypes were not specific to the disease. In the combined subject group, presence of disease was an obvious potential factor. This was largely substantiated by the volumetric comparison of bimodal regions.

5.4 Study IV

Study IV investigated correlations between grey and white matter volumes both within and across the posterior superior, posterior inferior, and anterior vermis. These correlations were quite strong in healthy control subjects, suggesting that a proper balance between the volumes of the different tissue types is important to healthy vermian constitution. The correlational structure was noticeably weaker in the patients, as shown by lowed or complete lack of significance in the correlations and increased variance in grey to white matter tissue ratios.

In light of the fact that there is no known direct connectivity between these three vermis regions, it is worth noting that correlations between regions tended to be quite strong in the controls. Correlations between grey matter in the posterior superior vermis and white matter in the anterior vermis and in the posterior inferior vermis were significant to over four places. The inter-regional correlation in the controls could reflect the presence of a larger brain circuit, possibly the vermis-thalamus-cortical network hypothesized by theories of cognitive dysmetria [3, 75]. Such a circuit may involve the entire vermis and requiring vermian input balanced among the three anatomical divisions. In the patients, the inter-regional correlations were the most affected while the intra-region correlations were relatively spared. Con-

tinuing the speculation just begin, this suggests that extra-vermian circuitry, if it exists, is deficient in the patients. It is impossible to say, however, if deficiency in neural circuitry causes aberrant vermian development or if the causality operates in the reverse direction.

Intra-region correlation was relatively spared in the patients. This shows that strong anatomical connectivity is associated with strong correlations between grey and white matter volumes, even in the presence of pathology. The pathology is evidenced by significantly increased grey to white matter ratios in two of the three vermian division, and significantly increased variance in these ratios in all of the divisions.

A possible weakness to the study is that the tissue segmentation is has not been validated for the vermis; this was mentioned in the manuscript. In defense of the study, the method is well validated for cortical regions and the strength of correlations in the control subjects suggests that it is valid also for the vermis.

An interesting continuation would be testing for relationships between disturbed proportions and cognitive skills. If found, this would provide strong evidence that proper balance between tissue types is a prerequisite for proper cognitive function. This research is a subject for a future study.

6 Concluding remarks

The studies included here have illustrated that machine learning based statistical analysis can make valid and useful contributions to medical knowledge. The results of Study I indicated that certain genetic loci may associate with increased risk of schizophrenia. Further investigation has confirmed these indications. The results of Study II indicate that volumes of the corpus callosum, putamen, caudate, and vermis better predict cognitive test scores than cortical grey matter volumes, and that volumetric/cognitive relationships are unchanged in schizophrenia. This supports speculation that subcortical and vermian abnormality form an important substrate of cognitive deficit in schizophrenia. The results of Study III showed that morphological subtypes of patients based on regional brain cortical thickness were unlikely, while finding that disease effects were pervasive in the cortex. This suggests that the heterogeneity of disease symptoms is not associated with different patterns of cortical thickness. The results of Study IV found a strong correlational structure between grey and white matter tissue volumes in the healthy vermis. It further found that that in patients with schizophrenia this correlational structure was weakened. It is hoped that the insights from these four studies will prove valuable to schizophrenia researchers.

7 Summary of findings

I. The following six genes contained SNPs showed association with increased risk of schizophrenia: BDNF, DRD2, NPY, NRG1, RELN, and SYN.

II. Relationships between brain structure volumes and cognitive skill were stronger for subcortical and cerebellar structures than for cortical structures. These relationships appeared unchanged in schizophrenia.

III. No evidence was found for patient subtypes based on brain cortical thickness. Patients had distinctively thinner cortex than controls in large areas of the frontal and temporal lobes, and some regions of the parietal lobe.

IV. The cerebellar vermis of healthy subjects had strong relationships between tissue volumes in and across anatomical divisions, and these relationships were weakened in patients with schizophrenia.

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