

Heritability Estimation and Risk Prediction in Schizophrenia

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Declaration

I declare that this thesis represents my own work, except where due acknowledgments is made, and that it has not been previously included in a thesis, dissertation or report submitted to this University or to any other institution for a degree, diploma or other qualification.

Signed.....

Acknowledgements

Abbreviations

CEU Northern Europeans from Utah. 39, 41, 42, 45, 46

CI confidence interval. 10

CNS central nervous system. 19

CNV copy number variation. 13, 14, 16, 18

DSM Diagnostic and Statistical Manual of Mental Disorders. 2

DZ dizygotic. 10

FGA First Generation Antipsychotic. 22, 23

GC Genomic Control. 17

GCTA Genome-wide Complex Trait Analysis. 16, 40, 42–44, 46–49

GD Gestation Day. 64

GO Gene Ontology. 66, 70, 71

GRM Genetic Relationship Matrix. 16

GWAS Genome Wide Association Study. 13, 14, 16–18, 27, 28, 41, 45

IL-6 Interleukin-6. 3

IQ intelligence quotient. 5

LD Linkage Disequilibrium. 12, 13, 17–19, 29, 33, 34, 36–43, 45, 46

LDSC LD SCore. 17, 19, 21, 27, 39, 40, 42–44, 46–49

LPS lipopolysaccharide. 3

maf Minor Allele Frequency. 5, 12, 41, 43, 44, 46, 48

MAGMA Multi-marker Analysis of GenoMic Annotation. 66, 71

- MHC** major histocompatibility complex. 14
- MIA** maternal immune activation. 3, 14
- MZ** monozygotic. 10, 11, 24
- NCP** non-centrality parameter. 34, 35
- NGS** next generation sequencing. 41
- NMDA** N-methyl-D-aspartate. 23
- PANSS** Positive and Negative Symptom Scale. 24
- PC** Principle Component. 66, 68
- PGC** Psychiatric Genomics Consortium. 13, 16, 66, 71
- PolyI:C** polyriboinosinic-polyribocytidilic acid. 3
- RIN** RNA integrity number. 64
- RPKM** Reads Per Kilobase per Million mapped reads. 64, 65, 67, 69
- SCZ** schizophrenia. 45, 47, 63
- SE** standard error. 18, 34
- SGA** Second Generation Antipsychotic. 23
- SHREK** SNP Heritability and Risk Estimation Kit. 27, 39, 40, 42–44, 46–50
- SNP** Single Nucleotide Polymorphism. 12, 13, 16–19, 28–30, 33, 38–48, 66
- SVD** Singular Value Decomposition. 37
- tSVD** Truncated Singular Value Decomposition. 37–39
- WGCNA** Weighted Gene Co-expression Network Analysis. 65, 66
- WHO** World Health Organization. 1, 2
- YLD** years lost due to disability. 1, 2

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Chapter 1

Introduction - Heritability Estimation in Schizophrenia

1.1 Schizophrenia

Schizophrenia is a detrimental psychiatric disorder, affecting around $0.3 \sim 0.7\%$ of the population(American Psychiatric Association, 2013). It is characterized by positive symptoms including delusions, hallucinations, disorganized speech and grossly disorganized behavior, and negative symptoms such as the diminished emotional expression(American Psychiatric Association, 2013) with a typical age of onset at late adolescent or late 20s in male and late 20s or early 30s in female(Schultz, North, and C. G. Shields, 2007).

Schizophrenia not only impose long lasting health, social and financial burden not only to the patients, but also to their families(Knapp, Mangalore, and Simon, 2004). Even more so, patients with schizophrenia increased suicide rate (Saha, Chant, and McGrath, 2007), leading to a higher mortality. Based on the World Health Organization (WHO) report, schizophrenia is one of the top 20 leading cause of years lost due to disability (YLD) in 2012, ranking 16 among all possible causes (table 1.1), demonstrating the extent of impact from schizophrenia to patients.

Due to the severity of schizophrenia, it has drawn much attention from the research community aiming to delineate the disease mechanics and be able to identify the risk factors. Arguably, the most important first step to any schizophrenia study is to have a robust and reliable disease diagnosis.

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Table 1.1: Top 20 leading cause of YLD calculated by WHO in year 2012. Schizophrenia was considered as one of the top 20 leading cause of YLD(World Health Organization, 2013)

Rank	Cause	YLD (000s)	% YLD	YLD per 100k population
0	All Causes	740,545	100	10466
1	Unipolar depressive disorders	76,419	10.3	1080
2	Back and neck pain	53,855	7.3	761
3	Iron-deficiency anaemia	43,615	5.9	616
4	Chronic obstructive pulmonary disease	30,749	4.2	435
5	Alcohol use disorders	27,905	3.8	394
6	Anxiety disorders	27,549	3.7	389
7	Diabetes mellitus	22,492	3	318
8	Other hearing loss	22,076	3	312
9	Falls	20,409	2.8	288
10	Migraine	18,538	2.5	262
11	Osteoarthritis	18,096	2.4	256
12	Skin diseases	15,744	2.1	223
13	Asthma	14,134	1.9	200
14	Road injury	13,902	1.9	196
15	Refractive errors	13,498	1.8	191
16	Schizophrenia	13,408	1.8	189
17	Bipolar disorder	13,271	1.8	188
18	Drug use disorders	10,620	1.4	150
19	Endocrine, blood, immune disorders	10,495	1.4	148
20	Gynecological diseases	10,227	1.4	145

1.2 Diagnosis

Schizophrenia was first named “Dementia Praecox” by Dr. Emil Kraepelin and was later renamed as schizophrenia by Dr. Eugen Bleuler(Jablensky, 2010). Early nosological entity for schizophrenia such as that in Diagnostic and Statistical Manual of Mental Disorders (DSM)-I and DSM-II were vague and unreliable where the inter-rater agreement can be as low as 54%.(Tsuang, Stone, and Faraone, 2000; Harvey et al., 2012)

Later nosologies addressed these problem by introducing structural assessment and clear defined criteria. With these improvements, the inter-rater agreement of DSM-III raised to $\sim 90\%$ (Harvey et al., 2012), suggesting the diagnosis were much more reliable.

Currently DSM is at its 5th edition(American Psychiatric Association, 2013). A patient will be diagnosed with schizophrenia(F20.9) if they suffered from 2 or more of the

1.3. RISK FACTORS OF SCHIZOPHRENIA

following symptoms for a significant portion of time during a 1-month period: 1) delusion; 2) hallucinations; 3) disorganized speech; 4) grossly disorganized or catatonic behaviour; and 5) negative symptoms such as diminished emotional expression, where one of the symptom must be either (1), (2) or (3). Signs of disturbance also need to persist for at least 6-month before the patient can be diagnosed with schizophrenia.

1.3 Risk Factors of Schizophrenia

Considerable effort has been made trying to identify possible risk factors of schizophrenia. It was first observed that there was an increased risk of schizophrenia in individual who were fetuses during the 1957 influenza epidemic(Mednick, 1958). Subsequently, other infectious agents such as HSV-2 and *T.gondii* were also found to increase the risk of schizophrenia if an individual's mother were infected during pregnancy. As different infectious agents all increase the risk of schizophrenia, it leads to the hypothesis of maternal immune activation (MIA) (Brown and Derkets, 2010). It was hypothesized that instead of a particular infectious agents, it was the maternal immune response that disrupt the brain development in the offspring, thus leading to an elevated risk of schizophrenia.

By utilizing the rodent models, it was found when the pregnant rodent was injected with the viral mimic polyriboinosinic-polyribocytidilic acid (PolyI:C) or the bacterial lipopolysaccharide (LPS), the offspring will display neuropathological features similar to those observed in schizophrenia(Urs Meyer, Joram Feldon, and Fatemi, 2009). It was further demonstrated that similar findings can be obtained through the injection of only the Interleukin-6 (IL-6)(Smith et al., 2007), suggesting that it was not the infection, but the maternal immune response that might have disrupted the fetal brain development.

Recent studies of global gene expression patterns in MIA-exposed rodent fetal brains (Garbett et al., 2012) suggest that MIA only causes a transient gene expression change in the fetal brain. Based on their observation, the author suggest that it was likely for the post-pubertal onset of schizophrenic and other psychosis-related phenotypes to be stemmed from attempts of the brain to counteract the environmental stress induced by MIA during its early development(Garbett et al., 2012).

Together, these results supports the involvement of MIA in the development of schizophrenia. It was even estimated that one third of all schizophrenia cases could have been prevented shall all infection were prevented from the entire pregnant population(Brown

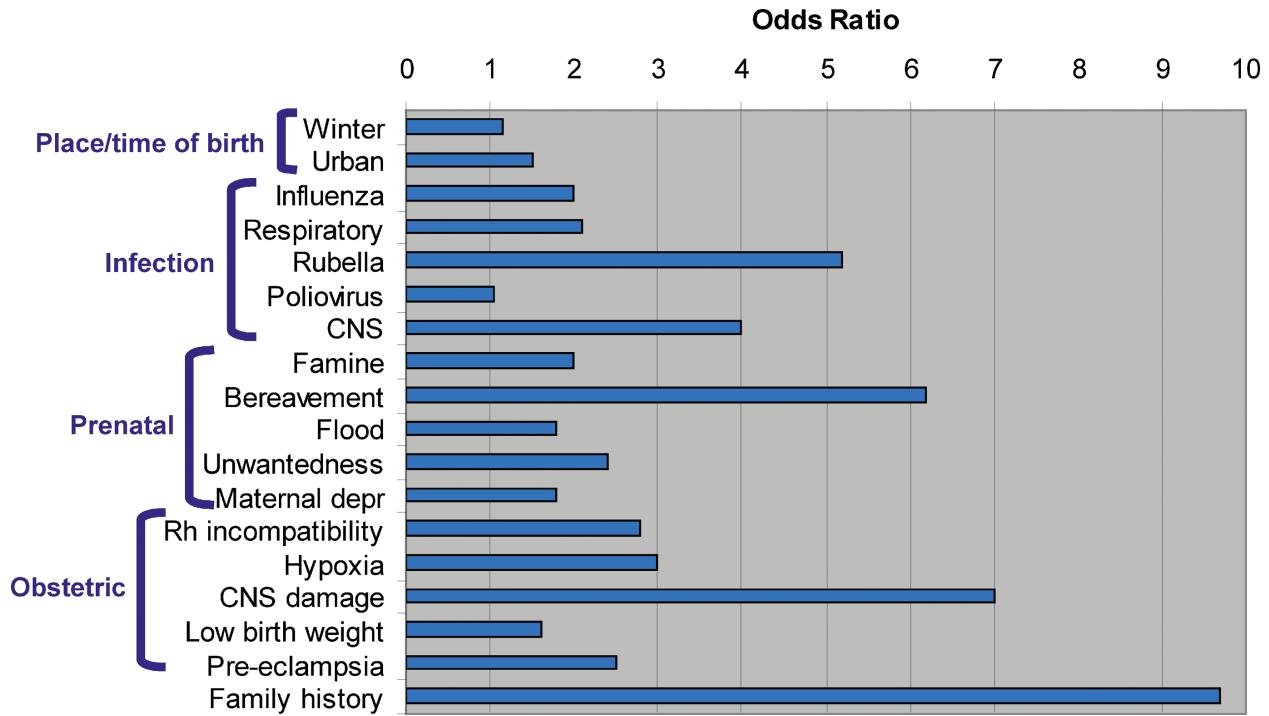


Figure 1.1: Risk factors of schizophrenia. It was observed that family history of schizophrenia was the largest risk factors. Risk of schizophrenia can be more than 9 times higher than the general population for individual with a family history of schizophrenia

and Derkits, 2010).

Similarly, tobacco consumption (Kelly and McCreadie, 1999), socio economical status and even the area of birth (e.g. urban vs suburb) were also found to be associated with increased risk of schizophrenia(McGrath et al., 2008). However, by and large, the single largest risk factor was family history of schizophrenia(fig. 1.1)(Sullivan, 2005). Studies conducted by Ernst Rüdin, Franz J. Kallmann and Hans Luxenburger, all demonstrated that the relatives of schizophrenia tends to have increased risk of schizophrenia(Irving I Gottesman and James Shields, 1982). The implication of such observation was two fold: as family members usually shares larger portion of their genetic effects with each other than that of the population, the genetic effects might be the main mediator of schizophrenia; on the other hand, culture, socio-economical status and area of birth usually also transmit within the family, so one cannot separate the environmental factors from the genetic factors.

It was important to study the relative contribution of genetic and environmental influence to individual differences in schizophrenia. If schizophrenia was indeed a genetic disease, we may then focus the resources into study of genetic variations in schizophrenia patients. To quantify the relative contribution of genetic and environmental influence, one

will need to estimate the *heritability* of schizophrenia.

1.4 Broad Sense Heritability

A key concept in quantitative genetics is *heritability*, which was defined as *proportion* of total variance of a trait in a population explained by variation of genetic factors in the population. One can partition observed phenotype into a combination of genetic and environmental components(Falconer and Mackay, 1996)

$$\text{Phenotype}(P) = \text{Genotype}(G) + \text{Environment}(E)$$

where the variance of the observed phenotype (σ_P^2) can be expressed as variance of genotype (σ_G^2) and variance of environment (σ_E^2)

$$\sigma_P^2 = \sigma_G^2 + \sigma_E^2$$

The broad sense heritability can then be defined as the ratio between the variance of the observed phenotype and the variance of the genetic effects

$$H^2 = \frac{\sigma_G^2}{\sigma_P^2}$$

One key feature of heritability is that it is a *ratio* of *populational* measurement at a specific time point. As a result of that, the heritability estimation might differ from one population to another due to difference in Minor Allele Frequency (maf) and one might obtain a different heritability estimate if the method or time-point of measurement of the trait differs because of different environmental factors coming into play. An classical example was the study of intelligence quotient (IQ) where the heritability estimation increases with age(Bouchard, 2013). It was hypothesize that the shared environment has a larger effect on individuals when they were young, and that as they become more independent, the effect of shared environment diminishes, leading to a *increased portion* of variance in IQ explained by the variance in genetic(Bouchard, 2013).

1.5 Narrow Sense Heritability

In reality, the problem of heritability was more complicated for there were different forms of genetic effects. For example, one can partition the genetic variance into variance of additive genetic effects (σ_A^2), variance of dominant genetic effects (σ_D^2) and other epistatic genetic effects (σ_I^2) such that

$$\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_I^2$$

where additive genetic variance was the variance explained by the average effects of all loci involved in the determination of the trait, whereas dominant genetic effects and epistatic genetic effectss were the interaction between alleles at the *same* locus or *different* loci respectively.

As individuals only transmit one copy of each allele to their offspring, relatives other than full siblings and identical twins will only share a maximum of one copy of the allele from each other. Considering that dominance and non-additive genetic effects were concerning the interactive effect, which usually involve more than one copy of the alleles, these effects are unlikely to contribute to the resemblance between relatives (Peter M Visscher, Hill, and Naomi R Wray, 2008). On the other hand, the additive genetic effects is usually transmitted from parent to offspring, thus it is usually more useful to consider the narrow sense heritability(h^2) which only consider the additive genetic effects:

$$\begin{aligned} h^2 &= \frac{\sigma_A^2}{\sigma_P^2} \\ h^2 &= \frac{\sigma_A^2}{\sigma_G^2 + \sigma_E^2} \end{aligned} \tag{1.1}$$

To obtain the additive genetic effect, we can first consider the genetic effect of parents to be $G_p = A + D$. As only half of the additive effect were transmitted to their offspring, the child will have a genetic effect of $G_c = \frac{1}{2}A + \frac{1}{2}A' + D'$ where A' is the additive genetic effect obtained from another parent by random and D' is the non-additive genetic effect in the offspring. If we then consider the parent offspring covariance, we will get

$$\begin{aligned} \text{Cov}_{OP} &= \sum \left(\frac{1}{2}A + \frac{1}{2}A' + D' \right) (A + D) \\ &= \frac{1}{2} \sum A^2 + \frac{1}{2} \sum AD + \frac{1}{2} \sum A'(A + D) + D'(A + D) \\ &= \frac{1}{2}V_A + \frac{1}{2}\text{Cov}_{AD} + \frac{1}{2}\text{Cov}_{A'A} + \frac{1}{2}\text{Cov}_{A'D} + \text{Cov}_{D'A} + \text{Cov}_{D'D} \end{aligned} \tag{1.2}$$

Under the assumption of random mating, A' should be independent from A and D . On the other hand, as D' was specific to the child, both of them should be independent from A and D . Moreover, the covariance between the additive genetics and non-additive genetics should be zero(Falconer and Mackay, 1996). Thus, eq. (1.2) becomes

$$\begin{aligned}\text{Cov}_{OP} &= \frac{1}{2}V_A + \text{Cov}_{AD} \\ &= \frac{1}{2}V_A\end{aligned}\tag{1.3}$$

Now if we assume the variance of phenotype of the parent and offspring were the same, then using eq. (1.3), we can obtain the narrow-sense heritability as

$$h^2 = \frac{1}{2} \frac{V_A}{\sigma_P^2}\tag{1.4}$$

If we consider the simple linear regression equation $Y = X\beta + \epsilon$, its slope can be calculated as

$$\beta_{XY} = \frac{\text{Cov}_{XY}}{\sigma_X Y}\tag{1.5}$$

which resemble eq. (1.4). Therefore, we can calculate the narrow sense heritability as

$$h^2 = 2\beta_{OP}\tag{1.6}$$

where β_{OP} is the slope of the simple linear regression regressing the phenotype of an offspring to the phenotype of *one* of its parents. We can further generalize eq. (1.6) to all possible relativity

$$h^2 = \frac{\beta_{XY}}{r}\tag{1.7}$$

where r is the relativity of X and Y .

A key assumption in this calculation was that the relatives does not share anything other than the additive genetic factors. However, this was usually not the case as relatives does tends to be in the same cultural group and might have similar socio-economical status which might all contribute the the variance of the trait. This might therefore lead to bias in eq. (1.7) and we shall discuss the partitioning of variance in the later sections.

Nonetheless, eq. (1.7) was still useful for the understanding of the calculation of heritability. However, in the case of discontinuous trait (e.g. disease status) the calculation becomes more completed because the variance of the phenotype was dependent on the population prevalence. As eq. (1.7) does not account for the trait prevalence, it cannot be directly applied to discontinuous traits. In order to perform heritability estimation, we will

need the concept of liability threshold model popularized by Falconer, 1965.

1.6 Liability Threshold

According the central limit theorem, if a phenotype is determined by a multitude of genetics and environmental factors with relatively small effect, then its distribution will likely follow a normal distribution as is the case of many quantitative traits(Peter M Visscher, Hill, and Naomi R Wray, 2008). The variance of phenotype can therefore be calculated as the variance under the normal distribution. However, such is not the case for disease such as schizophrenia where instead of having a continuous distribution of phenotype, only a dichotomous labeling of “affected” and “normal” were obtained. The variance of these phenotype were therefore more difficult to obtain.

Falconer (1965) proposed the liability threshold model, which suggesting that these discontinuous traits also follow a continuous distribution with an additional parameter called the “liability threshold”. Under the liability threshold model, the discontinuous traits were also affected by combination of multitude of genetics and environmental factors, each with a small effects, as in the case of the continuous traits. The main difference was that the phenotype of an individual is determined by whether if the combined effects of these factors(“liability”) were above a particular threshold (“liability threshold”). So for example, in the case of schizophrenia, only when an individual has a liability above the liability threshold will he/she be affected.

One can then estimate the heritability of the discontinuous by comparing the mean liability of the general population when compared to the relatives of the affected individuals. For example, if we consider a single threshold model of a dichotomous trait, where

T_G = Liability threshold of the general population

T_R = Liability threshold of relatives of the index case

q_G = Prevalence in the general population

q_R = Prevalence in relatives of the index case

L_a = Mean Liability of the index case

by assuming both the liability distribution of the general population and that of the relative of the index case both follows the standard normal distribution, we can align the two distribution with respect to T_G and T_R . We can then calculate the mean liability of the index case

1.7. TWIN STUDIES OF SCHIZOPHRENIA

L_a as $L_a = \frac{z_G}{q_G}$ where z_G is the density of the normal distribution at the liability threshold T_G . Then we can express the regression of relative's liability on the liability of the index case as

$$\beta = \frac{T_G - T_R}{L_a} \quad (1.8)$$

Thus, by applying eq. (1.8) to eq. (1.7), we get

$$h^2 = \frac{T_G - T_R}{L_a r} \quad (1.9)$$

1.7 Twin Studies of Schizophrenia

Now that we can deal with discontinuous traits, we shall come back to the limitation of eq. (1.7). The key limitation of eq. (1.7) was its inability to discriminate the genetic factors from the shared environmental factors. Such problem arise as family not only shared some of their genes, but they also tends to shared some of the environmental factors such as diet. In fact, this was the main reason for researchers to discord the argument that schizophrenia was a genetic disorder.

A classical adoption study carried out by Heston (1966) in 1966 set off to discriminate whether if the increased risk of schizophrenia in relatives of schizophrenia was caused by the shared environmental factors or the shared genetic factors. An advantages of adoption studies was that if the child was separated from their family early after birth, then the shared environmental factors should be minimized, thus any resemblance between the parent and child should be driven mainly by the shared genetic factors. Heston (1966) collected data of 47 individuals born from a schizophrenic mother during the period from 1915 to 1947. They were separated from their mother within three day of birth and were sent to a foster family. 50 matched control were also recruited to the study. It was observed that there was an increased risk of schizophrenia in individual born to schizophrenic mother when compared to the control group even-though they were brought up in a different environment as that of their mother. This result suggested that schizophrenia was likely driven by the shared genetic factors instead of the shared environmental factors.

Despite the usefulness of adoption studies in delineating the effect of shared environment from the genetic factors, collection of adoption data were difficult. Moreover, any prenatal influence such as alcohol abuse during pregnancy might confound the results.

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Therefore, an alternative way would be the twin studies using the relationship between the monozygotic (MZ) and dizygotic (DZ) twins.

Theoretically, MZ twins should share all their genetic components (both additive(A) and non-additive(D) genetic factors) and also their common environmental factors(C) where the only difference between a twin pair would be the non-shared environmental factors(E). As for the DZ twins, they should also share the same common environmental factors yet they only share $\frac{1}{2}$ of their additive genetic factors and $\frac{1}{4}$ of their non-additive genetic factors. The non-shared environmental was also by definition not shared among the twins(Rijdsdijk and Pak C Sham, 2002). Based on these assumptions, Falconer and Mackay, 1996 derived the heritability as

$$h^2 = 2(\rho_{MZ} - \rho_{DZ}) \quad (1.10)$$

where ρ_{MZ} and ρ_{DZ} were the phenotype correlation between the MZ twins and DZ twins respectively.

By combining Falconer's formula and the concept of liability threshold model, I I Gottesman and J. Shields (1967) estimated that the heritability of schizophrenia to be > 60% based on previously collected twin data, strongly suggesting schizophrenia as a genetic disorder. The result was further supported by one of the landmark meta-analysis study conducted by Sullivan, Kendler, and Neale, 2003. Based on data obtained from 12 published schizophrenia twin studies, the authors found that although there was a non-zero contribution of environmental influence on liability of schizophrenia (11%, confidence interval (CI)=3% – 19%), there was a much larger contribution from genetics (81%, CI=73% – 90%), further supporting that schizophrenia was largely mediated by the genetic factors.

Such findings were not limited to twin-studies but were also reported in large scale population based studies. A recent large scale population based study in Sweden population(Lichtenstein et al., 2009) also found that there was a large genetic contribution in schizophrenia (64%). Although the estimated heritability(64%(Lichtenstein et al., 2009) vs 81%(Sullivan, Kendler, and Neale, 2003)) differs between the two studies, they, there is no doubt that schizophrenia is highly heritable, leading to the initiative of genetic research in schizophrenia.

1.8 Genetic Analysis of Schizophrenia

1.8.1 Genetic Architecture of Schizophrenia

Studies on estimation of heritability of schizophrenia strongly support schizophrenia as a genetic disorder. However, little was known about the mechanism of schizophrenia nor the genetic architecture of the disorder. All data from adoption studies, twin studies and family studies shown that schizophrenia does not follow the Mendelian framework I (Gottesman and J. Shields, 1967; Irving I Gottesman and James Shields, 1982). Specifically, shall schizophrenia be a Mendelian disorder, then we would expect all MZ siblings of the proband to also suffer from schizophrenia. However, the life time morbid risk of monozygotic twins were only 48% (fig. 1.2) (I. Gottesman, 1991), making it unlikely for schizophrenia to follow a Mendelian pattern.

Based on these observations, I. Gottesman and J. Shields, 1967 proposed that schizophrenia follows a polygenic model where disease phenotype were determined by the additive effects from multiple genes. Thus, schizophrenia is a complex genetic disorder with complicated pattern of inheritance. Their hypothesis was supported by the calculation of Risch, 1990a by taking into account of different inheritance model and the life time morbid risk observed in relatives of affected individuals.

Another interesting conclusion from the calculation of Risch (1990a) was the effect size of individual locus. By comparing the observed life time morbid risk and the calculated risk from different models, Risch suggested that genetic models with a single locus with risk of 3.0 and with all other loci of small effect or models with two or three loci with risk of 2.0 were most consistent with the observed life time morbid risk of schizophrenia. (Risch,

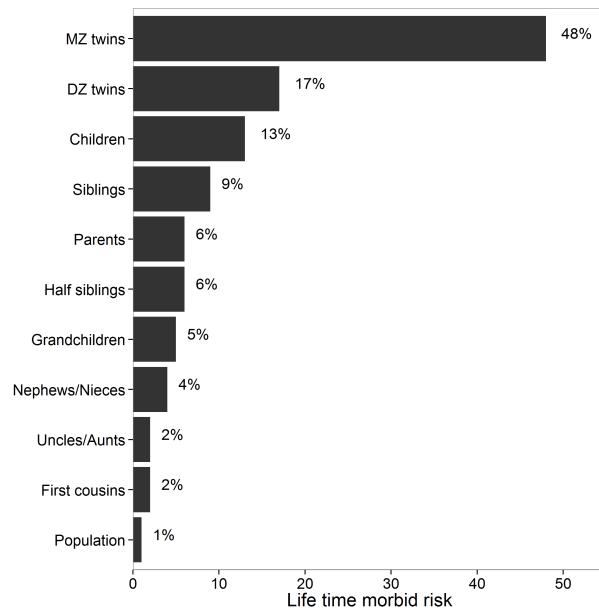


Figure 1.2: Lifetime morbid risks of schizophrenia in various classes of relatives of a proband. It was noted that the morbid risk of monozygotic (MZ) twins were only 48%, much lower than one would expect if schizophrenia follows a Mendelian pattern. Reproduced with permission from journal (Riley and Kendler, 2006).

1990b).

Risch's calculation provided an explanation for the early inconsistent findings of linkage studies in schizophrenia(Harrison and Weinberger, 2005). As linkage studies were aimed to identify genetic variation of large effect size they failed to capture genetic loci with small effect size. It was therefore tempting to suggest that schizophrenia only follows the "common disease-common variant" model, which stated that schizophrenia should be mediated by large amount of common variants such as Single Nucleotide Polymorphism, each carries a small effect size.

However, another possible hypothesis was that the variation mediating schizophrenia were rare, therefore require a large sample size to detect. The inconsistent results of the early linkage studies might be due to the inadequate sample size. This lead to some researchers suggesting the "common disease-rare variant" hypothesis, which propose that schizophrenia was mediated by a small amount of rare variants, each with a large effect size(McClellan, Susser, and King, 2007).

Nevertheless, success in genetic research of schizophrenia remains limited. Only until the initiation of Human Genome Project and the technological advance resulted from the it does genetic research of schizophrenia entered an era of success.

1.8.2 The Human Genome Project and HapMap Project

In 1990, the Human genome project was initiated, aiming at constructing the first physical map of the human genome at per nucleotide resolution(Lander et al., 2001). The completion of the human genome project has opened up a new era of genetic research, allowing researchers to identify Single Nucleotide Polymorphisms (SNPs) on the human genome, which is one of the major source of genetic variation.

Soon after the completion of the human genome project, the HapMap Project was initiated(Consortium, 2005), aiming to provide a genome-wide database of common human sequence variation such as SNPs with $\text{maf} \geq 0.05$. More importantly was that the HapMap Project also provided a detailed Linkage Disequilibrium (LD) map of the human genome.

LD was of particular importance to genetic research for it was the non-random correlation of genotypes between 2 genetic locus. SNPs in high LD were usually observed together in the human genome. When a large amount of SNPs were in high LD together, they form what was known as a LD block. By performing association testing on SNPs representing

a LD block(“tagging”), one can avoid the need of performing association on the whole genome, therefore reducing the cost of the experiment. This was the fundamental concept of Genome Wide Association Study (GWAS) which tests the which was now extensively used in the genetic research.

1.8.3 Genome Wide Association Study

In GWAS, genome-wide genotyping array were commonly used to systematically detect genetic variants such as SNP and copy number variation (CNV). For quantitative traits, the association between the trait and frequency of the variants were calculated using methods such as linear regression. On the other hand, for dichotomous traits such as schizophrenia, the frequency of the variants were compared between the case and control samples using methods such as chi-square test or logistic regression. Because of the problem of multiple testing, only variants with a p-value passing a genome wide threshold ($p\text{-value} \leq 5 \times 10^{-8}$) were considered significant. Another possible method to decide the significant threshold was to consider the “effective number” of tests(Li et al., 2011) taking into consideration of LD as not all tests in a GWAS were independent of each other. The power of the GWAS were determined by the magnitude of effect, sample size, and required level of statistical significance(the false-positive, or type I, error rate)(S. Purcell, Cherny, and P C Sham, 2003). A large sample size and a large effect usually result in a larger power.

Single Nucleotide Polymorphism

Despite the great promise from GWAS, early GWAS in schizophrenia remain largely disappointing and were unable to identify any robust genetic markers associated with schizophrenia. The failure of early GWAS in schizophrenia were mainly due to the relative small sample size of the studies, which result in low detection power.

To overcome the problem of small sample size, large consortium were formed such that data from different research groups from different countries were combined, essentially providing a large sample size for the analysis. By 2014, the Schizophrenia Working group of the Psychiatric Genomics Consortium (PGC) has collected 34,241 schizophrenia samples and 45,604 controls(Stephan Ripke et al., 2014). By combining the samples with those obtained by deCODE genetics, a total of 36,989 schizophrenia samples and 113,075 controls were used for the largest meta analysis of schizophrenia. In their study(Stephan Ripke et al., 2014), 128 linkage-disequilibrium-independent SNPs were found to exceeded the genome-

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wide significance($p\text{-value} \leq 5 \times 10^{-8}$), corresponding to 108 genetic loci. 75% of these loci contain protein coding genes and a further 8% of these loci were within 20kb of a gene. It was found that genes involved in glutamatergic neurotransmission (e.g. *GRM3*, *GRIN2A* and *GRIA1*), synaptic plasticity and genes encoding the voltage-gated calcium channel subunits (e.g. *CACNA1C*, *CACNB2* and *CACNA1I*) were among the genes associated within these loci. Importantly, *DRD2*, the target of all effective anti-psychotic drug were also associated with schizophrenia. This result converges with existing knowledge of *DRD2* being involved in the pathology of schizophrenia, supported by multiple lines of research(Talkowski et al., 2007). It was further demonstrated that schizophrenia association were significantly enriched at enhancers active in brain and enriched at enhancers active in tissues with important immune functions(fig. 1.3)(Stephan Ripke et al., 2014).

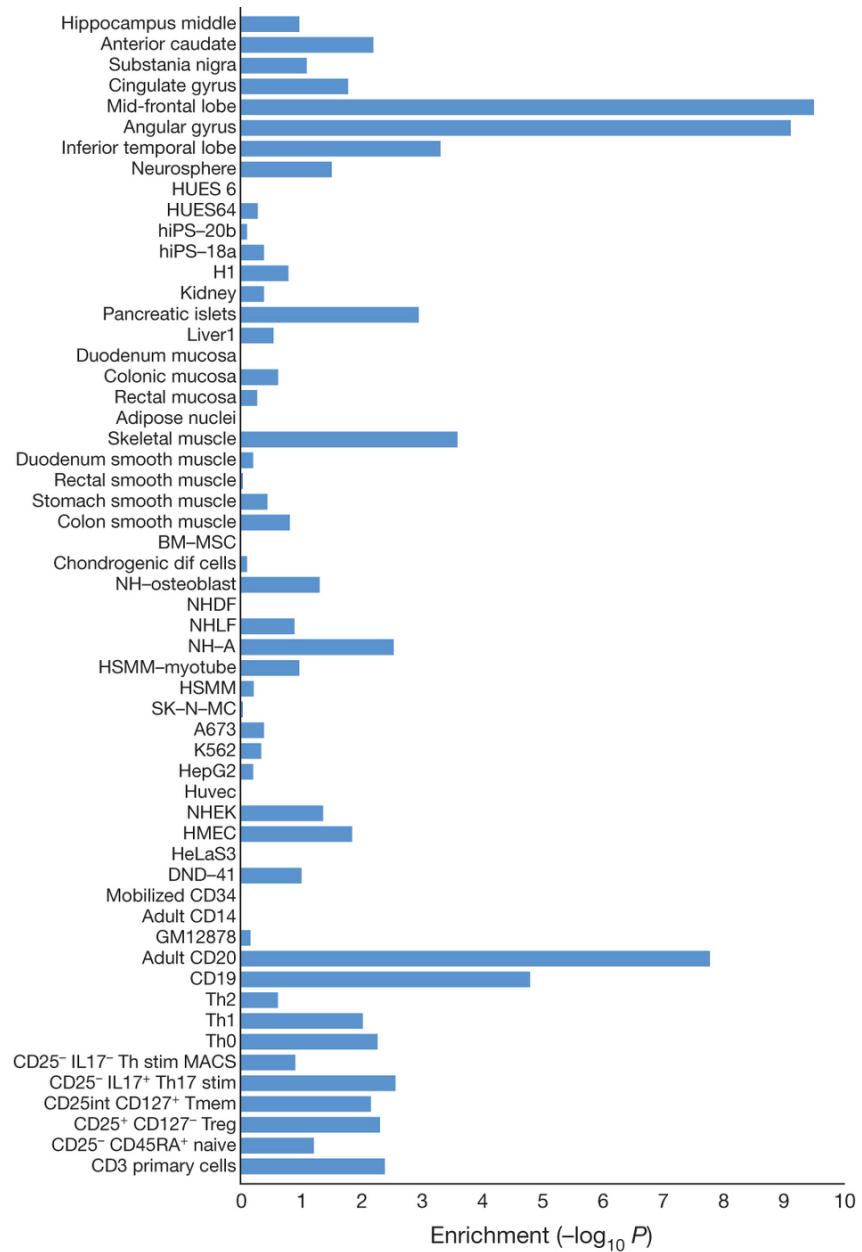
The enrichment of immune related enhancers remains significant even after the removal of major histocompatibility complex (MHC) region from the analysis, provided further genetic support of the involvement of the immune system in the etiology of schizophrenia. Because of its role in neural development(Zhao and Schwartz, 1998; Deverman and Patterson, 2009), it is likely that the perturbation in the immune system might disrupt the brain development, therefore increasing the risk of schizophrenia. Indeed, studies on MIA has demonstrated that cytokine imbalance might predispose individual to schizophrenia(U Meyer, J Feldon, and Yee, 2009).

Copy Number Variation

Another important arm of genetic research in schizophrenia was to identify copy number variation (CNV) associated with schizophrenia. CNV were classified as segment of DNA that is 1kb or larger and that is present at a different copy number when compared to the reference genome, usually in the form of insertion, deletion or duplication(Feuk, Carson, and Scherer, 2006). Due to the length of these variants, the CNV might contain the entire genes and their regulatory regions which might in turn contribute to significant phenotypic differences(Feuk, Carson, and Scherer, 2006).

To identify robust association between CNV and schizophrenia, Szatkiewicz et al., 2014 conducted a GWAS for CNV association with schizophrenia used the Swedish national sample (4,719 schizophrenia samples and 5,917 controls). In their study, they were able to association between schizophrenia and CNV such as 16p11.2 duplications, 22q11.2 deletions, 3q29 deletions and 17q12 duplications were identified. Through the gene set association analysis, calcium channel signaling and binding partners of the fragile X mental retardation

Figure 1.3: Enrichment of enhancers of SNPs associated with schizophrenia. It was observed that the largest enrichment were in cell lines related to the brain and in tissues with important immune functions. Graphs reproduced with permission from the journal.(Stephan Ripke et al., 2014)



protein were found to be associated with these CNV(Szatkiewicz et al., 2014). Interestingly, the calcium channel signaling were also enriched in the PGC GWAS on SNP association, suggesting that the variants were converging on similar set of pathway or gene sets.

Unlike the result form the GWAS on SNP data, the CNV identified were rare(≤ 12 in 4,719 samples) and has a relative large effect (e.g. 22q11 deletion has an odd ratio of 16.32(Szatkiewicz et al., 2014)). The results from the SNP GWAS supports the “common disease-common variant” model whereas the GWAS on CNV supports the “common disease-rare variant” model, illustrating the complex genetic model behind the etiology of schizophrenia.

Although the GWAS in schizophrenia seems to return a lot of interesting results, the question remains: How much of the known genetic risk factors associated explain the disease risk of schizophrenia? To answer these question, we need to estimate the heritability based on the GWAS data. However, in order to obtain the large volume of data, most of the samples were not relatives. How can one estimate the heritability based only on the genetic data of the general population instead of family or twin data?

1.8.4 Genome-wide Complex Trait Analysis

Unlike family based data, the relationship between the samples were unknown. Yet in a typical GWAS, the genotype of each individuals were known. The “genetic distance” between two individual will provide an estimate of their relationship, thus allowing the calculation of heritability. J Yang et al. (2011) use the concept of genetic distance to calculate the Genetic Relationship Matrix (GRM) to represent the relationship between individuals. The GRM were then used in the restricted maximum likelihood analysis(REML) to estimate the heritability of the trait(J Yang et al., 2011). This was implemented in Genome-wide Complex Trait Analysis (GCTA) and were now wildly used in the estimation of heritability on GWAS data.

The problem with GCTA was that it require the genotype data to estimate the heritability. However, for complex disease like schizophrenia, the data were usually obtained from multiple data source. Because of privacy issues, usually only the test statistic were shared among the research groups and only meta analysis were performed. Given there was no raw genotype data, it is impossible to calculate the GRM, thus making the use of GCTA impossible.

1.8.5 LD SCore

Sometimes, in a GWAS study, one can observe an general inflation of test statistics. It was usually considered to be contributed to the presence of confounding factors such as population stratification under the assumption that most of the SNPs should have no association to the disease. It was therefore a common practice for one to perform the Genomic Control (GC) on the GWAS results(Zheng, Freidlin, and Gastwirth, 2006).

The problem of the GC was that the basic assumption of a small number of causal SNPs might not be true. Through careful simulation, Jian Yang, Weedon, et al. (2011) demonstrated that in the absence of population stratification and other form of technical artifacts, the presence of polygenic inheritance can also inflate the test statistic(Jian Yang, Weedon, et al., 2011). More importantly, they observed that the magnitude of inflation was determined by the *heritability*, the LD structure, sample size and the number of causal SNPs of the trait.

Following on this observation, B. K. Bulik-Sullivan et al. (2015) developed the LD SCore (LDSC). The fundamental concept of LDSC was that the more genetic variant a SNP tag, the more likely for it to be able to tag a causal variant; whereas population stratification and cryptic relatedness should not be associated with LD. The number of genetic variants tagged by a SNP_j (l_j)(LD score) was then defined as the sum of r^2 of the k SNPs within a 1cM window of SNP_j:

$$l_j = \sum_k r_{jk}^2 \quad (1.11)$$

The expected χ^2 of SNP_j was then defined as a function of the LD score (l_j), the number of samples (N), the number of SNPs in the analysis(M), the contribution of confounding factors (a) and most importantly, the heritability (h^2):

$$\text{E}[\chi_j^2 | l_j] = \frac{Nl_j h^2}{M} + Na + 1 \quad (1.12)$$

If one express the LD score and the *chi*² as vectors \mathbf{L} and $\boldsymbol{\chi}^2$ respectively, eq. (1.12) becomes a regression of the χ^2 against the LD score:

$$\boldsymbol{\chi}^2 = \frac{N}{M} \mathbf{L} h^2 + Na + 1 \quad (1.13)$$

As a result of that, the heritability h^2 will be the slope of the regression and the intercept minus one will represent the mean contribution of the confounding bias such

as those of population stratification. Thus, eq. (1.13) can be used for the estimation of heritability given only the test statistics and the population LD were provided.

Using data from Stephan Ripke et al. (2014), and applying the liability threshold adjustment, B. K. Bulik-Sullivan et al. (2015) estimated the heritability of schizophrenia should be 0.555 with standard error (SE) of 0.008. The estimated heritability was lower than what was previously estimated from population based study(64%(Lichtenstein et al., 2009)) and twin studies(81%(Sullivan, Kendler, and Neale, 2003)). Possible reasons of such discrepancies might be that in Stephan Ripke et al. (2014)'s study, only SNPs data were collected. From Szatkiewicz et al. (2014), it was clearly demonstrated that other than SNPs, CNVs were also associated with schizophrenia. By ignoring CNVs in the estimation of heritability, the estimation of B. K. Bulik-Sullivan et al. (2015) would only provided a lower bound of heritability estimated. Another possibility of the “missing” heritability can be due to interaction between the genetic and environmental factors. Although previous studies(I I Gottesman and J. Shields, 1967) suggested that the non-additive genetic factors were unlikely to contribute to schizophrenia, the possibility of involvement of gene-environmental interaction $G \times E$ were not ruled out. Indeed, in the adoption study conducted by Tienari et al. (2004), it was found that individuals with higher genetic risk were significantly more sensitive to “adverse” vs “healthy” rearing patterns in adoptive families than are adoptees at low genetic risk(Tienari et al., 2004), providing support to a possible interaction between genetic and environmental factors. Therefore, in order to account for the “missing” heritability, one might need to consider genetic variations other than SNPs and might need to take into consideration of the $G \times E$ interaction.

Nonetheless, the heritability estimation from Stephan Ripke et al. (2014) were still encouraging, as for the first time in genetic research of schizophrenia, a large portion of heritability of schizophrenia were finally identified. This permit the genetic research of schizophrenia to move beyond statistical association and focus on the functional basis of the genetic susceptibility locus of schizophrenia.

1.8.6 Partitioning of Heritability of Schizophrenia

Traditionally, functional enrichment analysis in GWAS only take into account of SNPs that passed the genome wide significance threshold. However, for complex traits such as that of schizophrenia, much fo the heritability might lies in SNPs that do not reach genome wide significance threshold at the current sample size. For example, in 2013, only 13 risk

1.8. GENETIC ANALYSIS OF SCHIZOPHRENIA

loci were detected using 13,833 schizophrenia samples and 18,310 controls (S Ripke et al., 2013). When the sample size increased to 34,241 schizophrenia samples and 45,604 controls in 2014, 108 risk loci were identified(Stephan Ripke et al., 2014). Thus, if one only consider the significant loci, risk loci that have not reach genome wide significance threshold might be ignored from the analysis, decreasing the power of the functional enrichment analysis.

Unlike traditional functional enrichment analysis, LDSC uses information from all SNPs and taking into account of the LD structure to partition heritability into different functional categories. Thus should be more powerful when compared to traditional analysis and should help to provide useful insight into the disease etiology of schizophrenia.

Finucane et al. (2015) used data from Stephan Ripke et al. (2014) and functional categories derived from the ENCODE annotation(ENCODE Project Consortium, 2012), the NIH Roadmap Epigenomics Mapping Consortium annotation(Bernstein et al., 2010) and other studies(Finucane et al., 2015), it was found that the brain cell types were most enriched in schizophrenia, especially those related to the central nervous system (CNS). Of all the functional categories, the most enriched category in schizophrenia was the H3K4me3 mark in the fetal brain(table 1.2). As H3K4me3 was mostly linked to active promoters, it was likely for genes that were active in fetal brain (e.g genes related to brain development) to be associated with schizophrenia, supporting the idea of schizophrenia as an neuro-developmental disorder.

Moreover, it was also observed that the second most enriched cell types were those related to immunity. Undoubtedly, the CNS and the immune system have an important role in the disease etiology of schizophrenia.

Cell type	cell-type group	Mark	P-value
Fetal brain**	CNS	H3K4me3	3.09×10^{-19}
Mid frontal lobe**	CNS	H3K4me3	3.63×10^{-15}
Germinal matrix**	CNS	H3K4me3	2.09×10^{-13}
Mid frontal lobe**	CNS	H3K9ac	5.37×10^{-12}
Angular gyrus**	CNS	H3K4me3	1.29×10^{-11}
Inferior temporal lobe**	CNS	H3K4me3	1.70×10^{-11}
Cingulate gyrus**	CNS	H3K9ac	5.37×10^{-11}
Fetal brain**	CNS	H3K9ac	5.75×10^{-11}
Anterior caudate**	CNS	H3K4me3	2.19×10^{-10}
Cingulate gyrus**	CNS	H3K4me3	4.57×10^{-10}
Pancreatic islets**	Adrenal/Pancreas	H3K4me3	2.24×10^{-9}
Anterior caudate**	CNS	H3K9ac	3.16×10^{-9}
Angular gyrus**	CNS	H3K9ac	4.68×10^{-9}

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Mid frontal lobe**	CNS	H3K27ac	7.94×10^{-9}
Anterior caudate**	CNS	H3K4me1	1.20×10^{-8}
Inferior temporal lobe**	CNS	H3K4me1	3.72×10^{-8}
Psoas muscle**	Skeletal Muscle	H3K4me3	4.17×10^{-8}
Fetal brain**	CNS	H3K4me1	6.17×10^{-8}
Inferior temporal lobe**	CNS	H3K9ac	9.33×10^{-8}
Hippocampus middle**	CNS	H3K9ac	9.33×10^{-7}
Pancreatic islets**	Adrenal/Pancreas	H3K9ac	1.62×10^{-6}
Penis foreskin melanocyte primary**	Other	H3K4me3	2.09×10^{-6}
Angular gyrus**	CNS	H3K27ac	2.34×10^{-6}
Cingulate gyrus**	CNS	H3K4me1	2.82×10^{-6}
Hippocampus middle**	CNS	H3K4me3	2.82×10^{-6}
CD34 primary**	Immune	H3K4me3	4.68×10^{-6}
Sigmoid colon**	GI	H3K4me3	5.01×10^{-6}
Fetal adrenal**	Adrenal/Pancreas	H3K4me3	6.31×10^{-6}
Inferior temporal lobe**	CNS	H3K27ac	8.32×10^{-6}
Peripheral blood mononuclear primary**	Immune	H3K4me3	9.33×10^{-6}
Gastric**	GI	H3K4me3	1.17×10^{-5}
Substantia nigra*	CNS	H3K4me3	1.95×10^{-5}
Fetal brain*	CNS	H3K4me3	2.63×10^{-5}
Hippocampus middle*	CNS	H3K4me1	3.31×10^{-5}
Ovary*	Other	H3K4me3	6.46×10^{-5}
CD19 primary (UW)*	Immune	H3K4me3	7.08×10^{-5}
Small intestine*	GI	H3K4me3	8.51×10^{-5}
Lung*	Cardiovascular	H3K4me3	1.17×10^{-4}
Fetal stomach*	GI	H3K4me3	1.29×10^{-4}
Fetal leg muscle*	Skeletal Muscle	H3K4me3	1.51×10^{-4}
Spleen*	Immune	H3K4me3	1.70×10^{-4}
Breast fibroblast primary*	Connective/Bone	H3K4me3	2.04×10^{-4}
Right ventricle*	Cardiovascular	H3K4me3	2.14×10^{-4}
CD4+ CD25- Th primary*	Immune	H3K4me3	2.19×10^{-4}
CD4+ CD25- IL17- PMA Ionomycin stim MACS Th sprimary*	Immune	H3K4me1	2.19×10^{-4}
CD8 naive primary (UCSF-UBC)*	Immune	H3K4me3	2.24×10^{-4}
Pancreas*	Adrenal/Pancreas	H3K4me3	2.34×10^{-4}
CD4+ CD25- Th primary*	Immune	H3K4me1	2.75×10^{-4}
CD4+ CD25- CD45RA+ naive primary*	Immune	H3K4me1	2.75×10^{-4}
Colonic mucosa*	GI	H3K4me3	3.24×10^{-4}
Right atrium*	Cardiovascular	H3K4me3	3.31×10^{-4}
Fetal trunk muscle*	Skeletal Muscle	H3K4me3	3.39×10^{-4}

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CD4+ CD25int CD127+ Tmem primary*	Immune	H3K4me3	3.47×10^{-4}
Substantia nigra*	CNS	H3K9ac	3.63×10^{-4}
Placenta amnion*	Other	H3K4me3	4.17×10^{-4}
Breast myoepithelial*	Other	H3K9ac	5.50×10^{-4}
CD8 naive primary (BI)*	Immune	H3K4me1	5.75×10^{-4}
Substantia nigra*	CNS	H3K4me1	6.61×10^{-4}
Cingulate gyrus*	CNS	H3K27ac	7.94×10^{-4}
CD4+ CD25- CD45RA+ naive primary*	Immune	H3K4me3	8.71×10^{-4}

Table 1.2: Enrichment of Top Cell type of Schizophrenia. * = significant at False Discovery Rate < 0.05 . ** = significant at $p < 0.05$ after correcting for multiple hypothesis. Reproduce with permission from Journal.(Finucane et al., 2015)

1.8.7 Genetic Correlation

Another very important application of LDSC is that it allow one to identify the genetic correlation between traits(B. Bulik-Sullivan et al., 2015). The genetic correlation can be used as an genetic analogue to co-morbidity, thus allowing deeper understanding to the etiology of the traits. Above all, genetic correlation was important in studying the treatment response. It has been observed that there was an increased prevalence of anxiety, depression and substance abuse in schizophrenia(Buckley et al., 2009). These co-morbidity were generally associated with more severe psychopathology and with poorer outcome(Buckley et al., 2009). A deeper understanding of possible co-morbidity between different traits and schizophrenia might provide insight not only to the disease etiology of schizophrenia, it might even provide important information in possible treatment options for schizophrenia. Using breast cancer as an example, it was found that patients with comorbidity had poorer survival than those without comobidity(Søgaard et al., 2013) and it was suggested that by treating the comorbid diseases, one might be able to delay mortality in breast cancer patients(Ording et al., 2013).

By applying their method to 25 different phenotypes, B. Bulik-Sullivan et al. (2015) shown that schizophrenia has significant genetic correlation with bipolar disorder, major depression and more surprisingly, anorexia nervosa. Previous studies have always suggest there to be an co-morbidity between schizophrenia and bipolar disorder (Lichtenstein et al., 2009; S. M. Purcell et al., 2009; Buckley et al., 2009). Similarly, it was not uncommon for schizophrenia to display depressive symptoms(Buckley et al., 2009). It was even observed that individuals at high risk and ultrahigh risk for developing schizophrenia have generally

demonstrated a significant degree of depressive symptoms prior to and during the emergence of psychotic symptoms, suggesting a close relationship between schizophrenia and depression.

On the other hand, the genetic correlation between schizophrenia and anorexia nervosa were slightly unexpected for there has been a lack of study in the co-morbidity between eating disorder and schizophrenia. Nonetheless, this finding raises the possibility of similarity between anorexia and nervosa.

1.9 Antipsychotics

Despite the success in the genetic research of schizophrenia, an effective cure of schizophrenia was yet to be found. Currently, the main treatment method for schizophrenia was the use of antipsychotic drugs yet there was a large variability between individuals in their response to the drugs. Some might even suffer from adverse side effects such as agranulocytosis. Thus, it is important to administrate the optimal drug for each individual. However, there was an lack of understanding of the factors influencing the drug response, making it difficult, if not impossible, to develop a robust diagnostic test for selecting the most appropriate treatment for individuals. Therefore there is a pressing need for better understanding treatment response in schizophrenia.

1.9.1 History of Antipsychotic

Early research in treatment of schizophrenia largely follows a random trial and error process where methods such as insulin coma therapy were proposed(Lehmann and Ban, 1997). The first antipsychotic drug Chlorpromazine, a phenothiazine, were developed in early 1950s. Subsequently within a period of less than 10 years, 20 other antipsychotic phenothiazine were in development. Collectively, they were considered as the First Generation Antipsychotics (FGAs).

FGAs were found to be extremely effective in reducing the positive symptoms of schizophrenia such as delusions, hallucinations and disorganized thinking yet not only were they ineffective against negative and cognitive symptoms, they might cause acute extrapyramidal side effects such as parkinsonism, limiting the usefulness of FGAs(Tandon, 2007).

In 1966, a new drug, name Clozapine was introduced(Lehmann and Ban, 1997). Clozapine has been shown to not only effective in reducing the positive symptoms but also

the negative symptoms. On top of that, it was shown that there was a reduced likelihood of extrapyramidal side effects(Lehmann and Ban, 1997) making it an ideal treatment for schizophrenia. However, Clozapine were also found to be associated with the severe and potentially lethal adverse side effect, agranulocytosis(Alvir et al., 1993). Thus Clozapine was not used as a first line treatment of schizophrenia(Lieberman, 1996). A number of drugs were subsequently developed, aiming to have the same level of effectiveness as Clozapine and not having the adverse side effect. These were considered as the Second Generation Antipsychotics (SGAs) which includes risperidone, olanzapine and quetiapin. Although the SGAs tends to have lower risk for extrapyramidal side effects, they tends to be associated with weight gain, diabetes mellitus, hyperlipidemia etc.(Üçok and Gaebel, 2008)

1.9.2 Mechanism of Action of Antipsychotic

Despite the success of FGAs in reducing the positive symptoms of schizophrenia, it was unclear as to how the FGAs exert their effects. In 1963, it was found that the FGAs reduced the positive symptoms by blocking the dopamine receptors(Lehmann and Ban, 1997), leading to the development of the dopamine hypothesis. The early version of dopamine hypothesis suggested there were a hyperactive dopamine transmission in schizophrenia patients. The blocking of dopamine receptors might then reduce the concentration of dopamine in the brain, thus reducing the symptoms of schizophrenia(Howes and Kapur, 2009).

However, it was observed that although SGAs have a superior efficiency when compared to FGAs, they also have a lower affinity for and occupancy of at D₂ receptors. Specifically, it was found that the ratio between affinity of serotonin receptor (5-HT₂) to that of the D₂ receptor were significantly greater (15.8 times) for SGAs when compared to FGAs(Meltzer, 1991). Some SGAs such as haloperidol, olanzapine were also shown to have an effect to the glutamate system such as decreasing the levels in rat brain of specific subunits of the N-methyl-D-aspartate (NMDA) receptor(Meltzer, 2004). Other than the difference in affinity and target receptors the region of effect might also explain some of the difference in efficacy between FGAs and SGAs. For example, Clozapine preferentially target the mesolimbic dopamine system while sparing the nigrostriatal dopamine system(Gardner, Walker, and Paredes, 1993). Altogether, these evidence revolutionize the dopamine hypothesis which now suggest schizophrenia is a result of both “hypodopaminergia” in the prefrontal cortex and “hyperdopaminergia” in the straitum(Howes and Kapur, 2009), with a possible involvement of the glutamate system.

Through the study of antipsychotic drugs, one not only can understand the mechanics of the drug, but also provide insight into the disease etiology of schizophrenia. Moreover, it was of particular interest for one to identify possible factors affecting the efficacy of antipsychotic, thus providing a guideline for selecting the optimal drug for schizophrenia patients.

1.9.3 Positive and Negative Symptom Scale (PANSS)

In order to study the response of antipsychotic, it is important to have an objective scale to quantify the reduction of symptoms. The Positive and Negative Symptom Scale (PANSS)(Kay, Fiszbein, and Opler, 1987) were among one of the most commonly used to measure the core symptoms of schizophrenia and is composed of 3 subscales: positive, negative and general psychopathology. There were a total of 30 different symptoms included in PANSS and each symptoms were rated from 1 to 7, thus the minimal score for PANSS is 30. So to calculate the percentage reduction of PANSS, which represent a reduction in severity of symptoms, the reduction of PANSS will then be divided by the original PANSS minus 30:

$$\% \text{improvement} = \frac{\text{PANSS}_{\text{after}} - \text{PANSS}_{\text{before}}}{\text{PANSS}_{\text{before}} - 30} \times 100\%$$

1.9.4 Factors Associated with Antipsychotic Responses

Factors such as diet, smoking and concomitant medications were known to significantly affect metabolic enzyme activity rates, thus have an impact to antipsychotic treatment response(Arranz and Munro, 2011). On the other hand, clinical features such as treatment adherence and duration of illness; individual variation such as gender and ethnicity all influence the treatment efficacy(Arranz and Munro, 2011).

Moreover, there were also incidence report of concordance of response in MZ twin data(Vojvoda et al., 1996; Mata et al., 2001), suggesting a possible involvement of genetic factors in the antipsychotic response. However, most of these studies only consist of a single pair of MZ, making it impossible to calculate the heritability of antipsychotic response. Nonetheless, these studies did help to raise the question as to whether if genetic profile of an individual will provide an objective guidance for optimal drug selection. Most importantly, unlike the environmental factors which were usually difficult to collect and were relatively subjective, the genetic profile were relatively easy to collect and can provide a objective representation of an individual. Therefore even-though the heritability of antipsychotic response

were not known, interest in studying the genetic contribution in antipsychotic response were raising.

1.9.5 Pharmacogenetics and Pharmacogenomics

1.10 Chapter Summaries

Chapter 2

Heritability Estimation

2.1 Introduction

The development of LD Score has brought great prospect in estimating the heritability of complex disease for one can now estimate the heritability of a trait without requiring the rare genotype. However, as noted by the author of LDSC, when the number of causal variants were small, or when working on targeted genotype array, LDSC tends to have a larger standard error or might produce funky results(B. K. Bulik-Sullivan et al., 2015). Thus, there might be room for improvement of the method such that the performance of heritability estimation should be robust to all possible condition (e.g. number of causal SNPs, type of genotyping array).

In this chapter, we would introduce our method of heritability estimation using test static called SNP Heritability and Risk Estimation Kit (SHREK). We will compare the performance of SHREK and LDSC using simulation of different genetic architectures.

2.2 Methodology

The overall aims of this study is to develop a robust algorithm for the estimation of the narrow sense heritability using only the summary statistic from a GWAS study. The work in this chapter were done in collaboration with my colleagues who have kindly provide their support and knowledges to make this piece of work possible. Dr Johnny Kwan, Dr Mianxin Li and Professor Sham have helped to laid the framework of this study. Dr Timothy Mak

has derived the mathematical proof for our heritability estimation method. Miss Yiming Li, Dr Johnny Kwan, Dr Miaxin Li, Dr Timothy Mak and Professor Sham have helped with the derivation of the standard error of the heritability estimation. Dr Henry Leung has provided critical suggestions on the implementation of the algorithm.

2.2.1 Heritability Estimation

The narrow-sense heritability is defined as

$$h^2 = \frac{\text{Var}(X)}{\text{Var}(Y)}$$

where $\text{Var}(X)$ is the variance of the genotype and $\text{Var}(Y)$ is the variance of the phenotype. In a GWAS, regression were performed between the SNPs and the phenotypes, giving

$$Y = \beta X + \epsilon \tag{2.1}$$

where Y and X are the standardized phenotype and genotype respectively. ϵ is then the error term, accounting for the non-genetic elements contributing to the phenotype (e.g. Environment factors). Based on eq. (2.1), one can then have

$$\begin{aligned} \text{Var}(Y) &= \text{Var}(\beta X + \epsilon) \\ \text{Var}(Y) &= \beta^2 \text{Var}(X) \\ \beta^2 \frac{\text{Var}(X)}{\text{Var}(Y)} &= 1 \end{aligned} \tag{2.2}$$

β^2 is then considered as the portion of phenotype variance explained by the variance of genotype, which can also be considered as the narrow-sense heritability of the phenotype.

A challenge in calculating the heritability from GWAS data is that usually only the test-statistic or p-value were provided and one will not be able to directly calculate the heritability based on eq. (2.2). In order to estimation the heritability of a trait from the GWAS test-statistic, we first observed that when both X and Y are standardized, β^2 will be equal to the coefficient of determination (r^2). Then, based on properties of the Pearson product-moment correlation coefficient:

$$r = \frac{t}{\sqrt{n - 2 + t^2}} \tag{2.3}$$

where t follows the student-t distribution and n is the number of samples. One can then

obtain the r^2 by taking the square of eq. (2.3)

$$r^2 = \frac{t^2}{n - 2 + t^2} \quad (2.4)$$

It is observed that t^2 will follow the F-distribution and when n is big, t^2 will converge into χ^2 distribution.

When the effect size is small and n is big, r^2 will be approximately χ^2 distributed with mean ~ 1 . We can then approximate eq. (2.4) as

$$r^2 = \frac{\chi^2}{n} \quad (2.5)$$

and define the *observed* effect size of each SNP to be

$$f = \frac{\chi^2 - 1}{n} \quad (2.6)$$

When there are LD between each individual SNPs, the situation will become more complicated as each SNPs' observed effect will contains effect coming from other SNPs in LD with it.

$$f_{\text{observed}} = f_{\text{true}} + f_{\text{LD}} \quad (2.7)$$

To account for the LD structure, we first assume our phenotype \mathbf{Y} and genotype $\mathbf{X} = (X_1, X_2, \dots, X_m)^t$ are standardized and that

$$\begin{aligned} \mathbf{Y} &\sim f(0, 1) \\ \mathbf{X} &\sim f(0, \mathbf{R}) \end{aligned}$$

Where \mathbf{R} is the LD matrix between SNPs.

We can then express eq. (2.1) in matrix form:

$$\mathbf{Y} = \boldsymbol{\beta}^t \mathbf{X} + \epsilon \quad (2.8)$$

Definition of heritability will then become

$$\begin{aligned} \text{Heritability} &= \frac{\text{Var}(\boldsymbol{\beta}^t \mathbf{X})}{\text{Var}(\mathbf{Y})} \\ &= \text{Var}(\boldsymbol{\beta}^t \mathbf{X}) \end{aligned} \quad (2.9)$$

If we then assume now that $\boldsymbol{\beta} = (\beta_1, \beta_2, \dots, \beta_m)^t$ has distribution

$$\begin{aligned}\boldsymbol{\beta} &\sim f(0, H) \\ \mathbf{H} &= \text{diag}(\mathbf{h}) \\ \mathbf{h} &= (h_1^2, h_2^2, \dots, h_m^2)^t\end{aligned}$$

where \mathbf{H} is the variance of the true effect. It is shown that heritability can be expressed as

$$\begin{aligned}\text{Var}(\boldsymbol{\beta}^t \mathbf{X}) &= \mathbb{E}_X \text{Var}_{\beta|X}(\mathbf{X}^t \boldsymbol{\beta}) + \text{Var}_X \mathbb{E}_{(\beta|X)}(\boldsymbol{\beta}^2 \mathbf{X}) \\ &= \mathbb{E}_X(\mathbf{X}^t \boldsymbol{\beta} \boldsymbol{\beta}^t \mathbf{X}) \\ &= \mathbb{E}_X(\mathbf{X}^t \mathbf{H} \mathbf{X}) \\ &= \mathbb{E}(\mathbf{X})^t \mathbf{H} \mathbb{E}(\mathbf{X}) + \text{Tr}(\text{Var}(\mathbf{X} \mathbf{H})) \\ &= \text{Tr}(\text{Var}(\mathbf{X} \mathbf{H})) \\ &= \sum_i h_i^2\end{aligned}\tag{2.10}$$

Now if we consider the covariance between SNP i (X_i) and Y , we have

$$\begin{aligned}\text{Cov}(\mathbf{X}_i, \mathbf{Y}) &= \text{Cov}(\mathbf{X}_i, \boldsymbol{\beta}^t \mathbf{X} + \epsilon) \\ &= \text{Cov}(\mathbf{X}_i, \boldsymbol{\beta}^t \mathbf{X}) \\ &= \sum_j \text{Cov}(\mathbf{X}_i, \mathbf{X}_j) \boldsymbol{\beta}_j \\ &= \mathbf{R}_i \boldsymbol{\beta}_j\end{aligned}\tag{2.11}$$

As both X and Y are standardized, the covariance will equal to the correlation and we can define the correlation between SNP i and Y as

$$\rho_i = \mathbf{R}_i \boldsymbol{\beta}_j\tag{2.12}$$

In reality, the *observed* correlation usually contains error. Therefore we define the *observed* correlation to be

$$\hat{\rho}_i = \rho_i + \frac{\epsilon_i}{\sqrt{n}}\tag{2.13}$$

for some error ϵ_i . The distribution of the correlation coefficient about the true correlation ρ is approximately

$$\hat{\rho}_i \sim f(\rho_i, \frac{(1 - \rho^2)^2}{n})$$

By making the assumption that ρ_i is close to 0 for all i , we have

$$\begin{aligned} \text{E}(\epsilon_i | \rho_i) &\sim 0 \\ \text{Var}(\epsilon_i | \rho_i) &\sim 1 \end{aligned}$$

We then define our z -statistic and χ^2 -statistic as

$$\begin{aligned} z_i &= \hat{\rho}_i \sqrt{n} \\ \chi^2 &= z_i^2 \\ &= \hat{\rho}_i^2 n \end{aligned}$$

From eq. (2.13) and eq. (2.12), χ^2 can then be expressed as

$$\begin{aligned} \chi^2 &= \hat{\rho}^2 n \\ &= n(\mathbf{R}_i \boldsymbol{\beta}_j + \frac{\epsilon_i}{\sqrt{n}})^2 \end{aligned}$$

The expectation of χ^2 is then

$$\begin{aligned} \text{E}(\chi^2) &= n(\mathbf{R}_i \boldsymbol{\beta} \boldsymbol{\beta}^t \mathbf{R}_i + 2\mathbf{R}_i \boldsymbol{\beta} \frac{\epsilon_i}{\sqrt{n}} + \frac{\epsilon_i^2}{n}) \\ &= n\mathbf{R}_i \mathbf{H} \mathbf{R}_i + 1 \end{aligned}$$

To derive least square estimates of h_i^2 , we need to find \hat{h}_i^2 which minimizes

$$\begin{aligned} \sum_i (\chi_i^2 - \text{E}(\chi_i^2))^2 &= \sum_i (\chi_i^2 - (n\mathbf{R}_i \mathbf{H} \mathbf{R}_i + 1))^2 \\ &= \sum_i (\chi_i^2 - 1 - n\mathbf{R}_i \mathbf{H} \mathbf{R}_i)^2 \end{aligned}$$

If we define

$$f_i = \frac{\chi_i^2 - 1}{n} \tag{2.14}$$

we got

$$\begin{aligned} \sum_i (\chi_i^2 - \text{E}(\chi_i^2))^2 &= \sum_i (f_i - \mathbf{R}_i \mathbf{H} \mathbf{R}_i)^2 \\ &= \mathbf{f} \mathbf{f}^t - 2\mathbf{f}^t \mathbf{R}_{sq} \hat{\mathbf{h}} + \hat{\mathbf{h}}^t \mathbf{R}_{sq}^t \mathbf{R}_{sq} \hat{\mathbf{h}} \end{aligned} \tag{2.15}$$

where $\mathbf{R}_{sq} = \mathbf{R} \circ \mathbf{R}$. By differentiating eq. (2.15) w.r.t \hat{h} and set to 0, we get

$$\begin{aligned} 2\mathbf{R}_{sq}^t \mathbf{R}_{sq} \hat{h}^2 - 2\mathbf{R}_{sq} \mathbf{f} &= 0 \\ \mathbf{R}_{sq} \hat{h}^2 &= \mathbf{f} \end{aligned} \quad (2.16)$$

And the heritability is then defined as

$$\text{Heritability} = \mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f} \quad (2.17)$$

2.2.2 Calculating the standard error

From eq. (2.17), we can derive the variance of heritability H as

$$\begin{aligned} \text{Var}(H) &= \text{E}[H^2] - \text{E}[H]^2 \\ &= \text{E}[(\mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f})^2] - \text{E}[\mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f}] (\text{E}[\mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f}])^t \\ &= \text{E}[\mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f} \mathbf{f}^t \mathbf{R}_{sq}^{-1} \mathbf{1}] - \text{E}[\mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f}] (\text{E}[\mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f}])^t \\ &= \mathbf{1}^t \mathbf{R}_{sq}^{-1} \text{E}[\mathbf{f} \mathbf{f}^t] \mathbf{R}_{sq}^{-1} \mathbf{1} - \text{E}[\mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f}] (\text{E}[\mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f}])^t \\ &= \mathbf{1}^t \mathbf{R}_{sq}^{-1} \text{Var}(\mathbf{f}) \mathbf{R}_{sq}^{-1} \mathbf{1} + \text{E}[\mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f}] (\text{E}[\mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f}])^t - \text{E}[\mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f}] (\text{E}[\mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f}])^t \\ &= \mathbf{1}^t \mathbf{R}_{sq}^{-1} \text{Var}(\mathbf{f}) \mathbf{R}_{sq}^{-1} \mathbf{1} \end{aligned} \quad (2.18)$$

Therefore, to obtain the variance of H , we first need to calculate the variance covariance matrix of \mathbf{f} .

We first consider the standardized genotype X_i with standard normal mean z_i and non-centrality parameter μ_i , we have

$$\begin{aligned} \text{E}[X_i] &= \text{E}[z_i + \mu_i] \\ &= \mu_i \\ \text{Var}(X_i) &= \text{E}[(z_i + \mu_i)^2] + \text{E}[(z_i + \mu_i)]^2 \\ &= \text{E}[z_i^2 + \mu_i^2 + 2z_i\mu_i] + \mu_i^2 \\ &= 1 \\ \text{Cov}(X_i, X_j) &= \text{E}[(z_i + \mu_i)(z_j + \mu_j)] - \text{E}[z_i + \mu_i]\text{E}[z_j + \mu_j] \\ &= \text{E}[z_iz_j + z_i\mu_j + \mu_iz_j + \mu_i\mu_j] - \mu_i\mu_j \\ &= \text{E}[z_iz_j] + \text{E}[z_i\mu_j] + \text{E}[z_j\mu_i] + \text{E}[\mu_i\mu_j] - \mu_i\mu_j \\ &= \text{E}[z_iz_j] \end{aligned}$$

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As the genotypes are standardized, therefore $\text{Cov}(X_i, X_j) == \text{Cor}(X_i, X_j)$, we can obtain

$$\text{Cov}(X_i, X_j) = \text{E}[z_i z_j] = R_{ij}$$

where R_{ij} is the LD between SNP_i and SNP_j. Given these information, we can then calculate $\text{Cov}(\chi_i^2, \chi_j^2)$ as:

$$\begin{aligned} \text{Cov}(X_i^2, X_j^2) &= \text{E}[(z_i + \mu_i)^2(z_j + \mu_j)^2] - \text{E}[z_i + \mu_i]\text{E}[z_j + \mu_j] \\ &= \text{E}[(z_i^2 + \mu_i^2 + 2z_i\mu_i)(z_j^2 + \mu_j^2 + 2z_j\mu_j)] - \text{E}[z_i^2 + \mu_i^2 + 2z_i\mu_i]\text{E}[z_j^2 + \mu_j^2 + 2z_j\mu_j] \\ &= \text{E}[(z_i^2 + \mu_i^2 + 2z_i\mu_i)(z_j^2 + \mu_j^2 + 2z_j\mu_j)] - (\text{E}[z_i^2] + \text{E}[\mu_i^2] + 2\text{E}[z_i\mu_i])(\text{E}[z_j^2] + \text{E}[\mu_j^2] + 2\text{E}[z_j\mu_j]) \\ &= \text{E}[z_i^2(z_j^2 + \mu_j^2 + 2z_j\mu_j) + \mu_i^2(z_j^2 + \mu_j^2 + 2z_j\mu_j) + 2z_i\mu_i(z_j^2 + \mu_j^2 + 2z_j\mu_j)] - (1 + \mu_i^2)(1 + \mu_j^2) \\ &= \text{E}[z_i^2(z_j^2 + \mu_j^2 + 2z_j\mu_j)] + \mu_i^2\text{E}[z_j^2 + \mu_j^2 + 2z_j\mu_j] + 2\mu_i\text{E}[z_i(z_j^2 + \mu_j^2 + 2z_j\mu_j)] - (1 + \mu_i^2)(1 + \mu_j^2) \\ &= \text{E}[z_i^2z_j^2 + z_i^2\mu_j^2 + 2z_i^2z_j\mu_j] + \mu_i^2 + \mu_i^2\mu_j^2 + 2\mu_i\text{E}[z_i z_j^2 + z_i\mu_j^2 + 2z_i z_j\mu_j] - (1 + \mu_i^2)(1 + \mu_j^2) \\ &= \text{E}[z_i^2z_j^2] + \mu_j^2 + \mu_i^2 + \mu_i^2\mu_j^2 + 4\mu_i\mu_j\text{E}[z_i z_j] - (1 + \mu_i^2 + \mu_j^2 + \mu_i\mu_j) \\ &= \text{E}[z_i^2z_j^2] + 4\mu_i\mu_j\text{E}[z_i z_j] - 1 \end{aligned}$$

Remember that $\text{E}[z_i z_j] = R_{ij}$, we then have

$$\text{Cov}(X_i^2, X_j^2) = \text{E}[z_i^2z_j^2] + 4\mu_i\mu_jR_{ij} - 1$$

By definition,

$$z_i|z_j \sim N(\mu_i + R_{ij}(z_j - \mu_j), 1 - R_{ij}^2)$$

We can then calculate $\text{E}[z_i^2z_j^2]$ as

$$\begin{aligned} \text{E}[z_i^2z_j^2] &= \text{Var}[z_i z_j] + \text{E}[z_i z_j]^2 \\ &= \text{E}[\text{Var}(z_i z_j|z_i)] + \text{Var}[\text{E}[z_i z_j|z_i]] + R_{ij}^2 \\ &= \text{E}[z_j^2\text{Var}(z_i|z_j)] + \text{Var}[z_j\text{E}[z_i|z_j]] + R_{ij}^2 \\ &= (1 - R_{ij}^2)\text{E}[z_j^2] + \text{Var}(z_j(\mu_i + R_{ij}(z_j - \mu_j))) + R_{ij}^2 \\ &= (1 - R_{ij}^2) + \text{Var}(z_j\mu_i + R_{ij}z_j^2 - \mu_jz_jR_{ij}) + R_{ij}^2 \\ &= 1 + \mu_i^2\text{Var}(z_j) + R_{ij}^2\text{Var}(z_j^2) - \mu_j^2R_{ij}^2\text{Var}(z_j) \\ &= 1 + 2R_{ij}^2 \end{aligned}$$

As a result, the variance covariance matrix of the χ^2 variances represented as

$$\text{Cov}(X_i^2, X_j^2) = 2R_{ij}^2 + 4R_{ij}\mu_i\mu_j \quad (2.19)$$

Considering that we only have the *observed* expectation, we should re-define eq. (2.19) as

$$\text{Cov}(X_i^2, X_j^2) = \frac{2R_{ij}^2 + 4R_{ij}\mu_i\mu_j}{n^2} \quad (2.20)$$

where n is the sample size.

By substituting eq. (2.20) into eq. (2.18), we will get

$$\text{Var}(H) = \mathbf{1}^t \mathbf{R}_{sq}^{-1} \frac{2\mathbf{R}_{sq} + 4\mathbf{R} \circ \mathbf{z}\mathbf{z}^t}{n^2} \mathbf{R}_{sq}^{-1} \mathbf{1} \quad (2.21)$$

where $\mathbf{z} = \sqrt{\chi^2}$ from eq. (2.14), with the direction of effect as its sign and \circ is the element-wise product (Hadamard product).

Problem with eq. (2.21) were that not only does it requires the direction of effect, the error in the LD matrix also tends to amplify due to its predominant role in the equation, leading to un-stable estimation of the SE.

Another way to get the SE is based on the fact that \mathbf{f} is approximately χ^2 distributed. Therefore eq. (2.16) can be viewed as a decomposition of a vector of χ^2 distributions with degree of freedom of 1. Replacing the vector \mathbf{f} with a vector of 1, we can perform the decomposition of the degree of freedom, getting the “effective number”(e) of the association(Li et al., 2011). Substituting e into the variance equation of non-central χ^2 distribution will yield

$$\text{Var}(H) = \frac{2(e + 2H)}{n^2} \quad (2.22)$$

eq. (2.22) will gives us an heuristic estimation of the SE.

2.2.3 Case Control Studies

When dealing with case control data, as the phenotype were usually discontinuous, we cannot directly use eq. (2.17) to estimate the heritability. Instead, we will need to employ the concept of liability threshold model from section 1.6.

Based on the derivation of Jian Yang, Naomi R. Wray, and Peter M. Visscher, 2010, the approximate ratio between the non-centrality parameter (NCP) obtained from case control studies (NCP_{CC}) and quantitative trait studies(NCP_{QT}) were

$$\frac{NCP_{CC}}{NCP_{QT}} = \frac{i^2 v(1 - v) N_{CC}}{(1 - K)^2 N_{QT}} \quad (2.23)$$

where

K = Population Prevalence

v = Proportion of Cases

N = Total Number of Samples

$$i = \frac{z}{K}$$

z = height of standard normal curve at truncation pretained to K

Based on this observation, we can then directly transform the NCP between the case control studies and quantitative trait studies. During the transformation, N_{CC} and N_{QT} will be the same, therefore eq. (2.23) became

$$NCP_{QT} = \frac{NCP_{CC}(1 - K)^2}{i^2 v(1 - v)} \quad (2.24)$$

By combining eq. (2.24) and eq. (2.14), we can then have

$$f = \frac{(\chi_{CC}^2 - 1)(1 - K)^2}{ni^2 v(1 - v)} \quad (2.25)$$

where χ_{CC}^2 is the test statistic from the case control association test. Finally, the heritability estimation of case control studies can be simplified to

$$\hat{\text{Heritability}} = \frac{(1 - K)^2}{i^2 v(1 - v)} \mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f} \quad (2.26)$$

2.2.4 Extreme Phenotype Selections

When extreme phenotype selection were performed, the variance of the selected phenotype will not be representative of that in the population. Most notably, the variance of the post selection phenotype will tends to increase. Thus, to adjust for this bias, one can multiple the estimated heritability \hat{h}^2 by the ratio between the variance before V_P and after $V_{P'}$ the selection process(Pak C Sham and S. M. Purcell, 2014):

$$\hat{\text{Heritability}} = \frac{V_{P'}}{V_P} \mathbf{1}^t \mathbf{R}_{sq}^{-1} \mathbf{f} \quad (2.27)$$

2.2.5 Calculating the Linkage Disequilibrium matrix

To estimate the heritability, the population LD matrix is required. In reality, one can only obtain the LD matrix based on a subset of the population (e.g. the 1000 genome project(Project et al., 2012) or the HapMap project(Altshuler et al., 2010)). There are therefore sampling errors among the LD elements.

Now if we consider eq. (2.17), the \mathbf{R}_{sq} matrix is required. As the squared LD is used, a positive bias is induced into our \mathbf{R}_{sq} matrix.

Based on Shieh (2010), one can correct for bias in the Pearson correlation ρ using

$$\rho = \rho \left\{ 1 + \frac{1 - \rho^2}{2(N - 4)} \right\} \quad (2.28)$$

where N is the number of sample used in the calculation of ρ . Similarly, there exists a bias correction equation for ρ^2 :

$$\rho^2 = 1 - \frac{N - 3}{N - 2}(1 - \rho^2) \left\{ 1 + \frac{2(1 - \rho^2)}{N - 3.3} \right\} \quad (2.29)$$

Therefore, we corrected the \mathbf{R}_{sq} based on eq. (2.29) such that the bias in estimation can be minimized.

2.2.6 Inverse of the Linkage Disequilibrium matrix

In order to obtain the heritability estimation, we will require to solve eq. (2.17). If \mathbf{R}_{sq} is of full rank and positive semi-definite, it will be straight-forward to solve the matrix equation. However, more often than not, the LD matrix are rank-deficient and suffer from multicollinearity, making it ill-conditioned, therefore highly sensitive to changes or errors in the input. To be exact, we can view eq. (2.17) as calculating the sum of $\hat{\mathbf{h}}^2$ from eq. (2.16). This will involve solving for

$$\hat{\mathbf{h}}^2 = \mathbf{R}_{sq}^{-1} \mathbf{f} \quad (2.30)$$

where an inverse of \mathbf{R}_{sq} is observed.

In normal circumstances (e.g. when \mathbf{R}_{sq} is full rank and positive semi-definite), one can easily solve eq. (2.30) using the QR decomposition or LU decomposition. However, when \mathbf{R}_{sq} is ill-conditioned, the traditional decomposition method will fail. Even if the decomposition is successfully performed, the result tends to be a meaningless approximation

to the true $\hat{\mathbf{h}}^2$.

Therefore, to obtain a meaningful solution, regularization techniques such as the Tikhonov Regularization (also known as Ridge Regression) and Truncated Singular Value Decomposition (tSVD) has to be performed(Neumaier, 1998). There are a large variety of regularization techniques, yet the discussion of which is beyond the scope of this study. In this study, we will focus on the use of tSVD in the regularization of the LD matrix. This is because the Singular Value Decomposition (SVD) routine has been implemented in the EIGEN C++ library (Guennebaud and Jacob, 2010), allowing us to implement the tSVD method without much concern with regard to the detail of the algorithm.

To understand the problem of the ill-conditioned matrix and regularization method, we consider the matrix equation $\mathbf{Ax} = \mathbf{B}$ where \mathbf{A} is ill-conditioned or singular with $n \times n$ dimension. The SVD of \mathbf{A} can be expressed as

$$\mathbf{A} = \mathbf{U}\Sigma\mathbf{V}^t \quad (2.31)$$

where \mathbf{U} and \mathbf{V} are both orthogonal matrix and $\Sigma = \text{diag}(\sigma_1, \sigma_2, \dots, \sigma_n)$ is the diagonal matrix of the *singular values* (σ_i) of matrix \mathbf{A} . Based on eq. (2.31), we can get the inverse of \mathbf{A} as

$$\mathbf{A}^{-1} = \mathbf{V}\Sigma^{-1}\mathbf{U}^t \quad (2.32)$$

Where $\Sigma^{-1} = \text{diag}(\frac{1}{\sigma_1}, \frac{1}{\sigma_2}, \dots, \frac{1}{\sigma_n})$. Now if we consider there to be error within \mathbf{B} such that

$$\hat{\mathbf{B}}_i = \mathbf{B}_i + \epsilon_i \quad (2.33)$$

we can then represent $\mathbf{Ax} = \mathbf{B}$ as

$$\begin{aligned} \mathbf{Ax} &= \hat{\mathbf{B}} \\ \mathbf{U}\Sigma\mathbf{V}^t\mathbf{x} &= \hat{\mathbf{B}} \\ \mathbf{x} &= \mathbf{V}\Sigma^{-1}\mathbf{U}^t\hat{\mathbf{B}} \end{aligned} \quad (2.34)$$

A matrix \mathbf{A} is considered as ill-condition when its condition number $\kappa(\mathbf{A})$ is large or singular when its condition number is infinite. One can represent the condition number as $\kappa(\mathbf{A}) = \frac{\sigma_1}{\sigma_n}$. Therefore it can be observed that when σ_n is tiny, \mathbf{A} is likely to be ill-conditioned and when $\sigma_n = 0$, \mathbf{A} will be singular.

One can also observe from eq. (2.34) that when the singular value σ_i is small, the error ϵ_i in eq. (2.33) will be drastically magnified by a factor of $\frac{1}{\sigma_i}$. Making the system of

equation highly sensitive to errors in the input.

To obtain a meaningful solution from this ill-conditioned/singular matrix \mathbf{A} , we may perform the tSVD method to obtain a pseudo inverse of \mathbf{A} . Similar to eq. (2.31), the tSVD of \mathbf{A} can be represented as

$$\mathbf{A}^+ = \mathbf{U}\Sigma_k\mathbf{V}^t \quad \text{and} \quad \Sigma_k = \text{diag}(\sigma_1, \dots, \sigma_k, 0, \dots, 0) \quad (2.35)$$

where Σ_k equals to replacing the smallest $n - k$ singular value replaced by 0 (Hansen, 1987). Alternatively, we can define

$$\sigma_i = \begin{cases} \sigma_i & \text{for } \sigma_i \geq t \\ 0 & \text{for } \sigma_i < t \end{cases} \quad (2.36)$$

where t is the tolerance threshold. Any singular value σ_i less than the threshold will be replaced by 0.

By selecting an appropriate t , tSVD can effectively regularize the ill-conditioned matrix and help to find a reasonable approximation to x . A problem with tSVD however is that it only work when matrix \mathbf{A} has a well determined numeric rank(Hansen, 1987). That is, tSVD work best when there is a large gap between σ_k and σ_{k+1} . If a matrix has ill-conditioned rank, then $\sigma_k - \sigma_{k+1}$ will be small. For any threshold t , a small error can change whether if σ_{k+1} and subsequent singular values should be truncated, leading to unstable results.

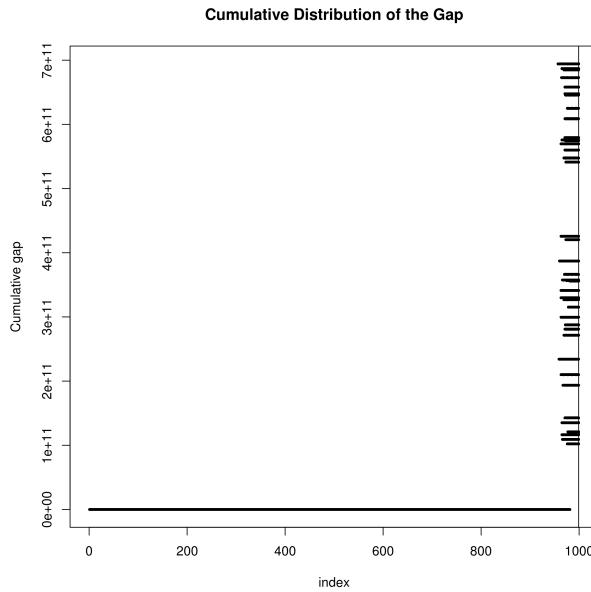
According to Hansen (1987), matrix where its rank has meaning will have well defined rank. As LD matrix is the correlation matrix between each individual SNPs, the rank of the LD matrix is the maximum number of linear independent SNPs in the region, therefore likely to have a well-defined rank. The easiest way to test whether if the threshold t and whether if the matrix \mathbf{A} has well-defined rank is to calculate the “gap” in the singular value:

$$gap = \sigma_k / \sigma_{k+1} \quad (2.37)$$

a large gap usually indicate a well-defined gap.

In this study, we adopt the threshold as defined in MATLAB, NumPy and GNU Octave: $t = \epsilon \times \max(m, n) \times \max(\Sigma)$ where ϵ is the machine epsilon (the smallest number a machine can define as non-zero). And we perfomed a simulation study to investigate the performance of tSVD under the selected threshold. Ideally, if the “gap” is large under the selected threshold, then tSVD will provide a good regularization to the equation.

Figure 2.1: Cumulative Distribution of “gap” of the LD matrix, the vertical line indicate the full rank. It can be observed that there is a huge increase in “gap” before full rank is achieved. Suggesting that the rank of the LD matrix is well defined



1,000 samples were randomly simulated from the HapMap(Altshuler et al., 2010) CEU population with 1,000 SNPs randomly select from chromosome 22. The LD matrix and its corresponding singular value were calculated. The whole process were repeated 50 times and the cumulative distribution of the “gap” of singular values were plotted (fig. 2.1). It is clearly show that the LD matrix has a well-defined rank with a mean of maximum “gap” of 466,198,939,298. Therefore the choice of tSVD for the regularization is appropriate.

By employing the tSVD as a method for regularization, we were able to solve the ill-posed eq. (2.16), and obtain the estimated heritability.

2.2.7 Comparing with LD SCore

Conceptually, the fundamental hypothesis of LDSC and SHREK were quite different. LDSC were based on the “global” inflation of test statistic and its relationship to the LD pattern and by using this relationship, LDSC might estimate the heritability. On the other hand, SHREK focuses more on the per-SNP level. Our main idea with SHREK was that the test statistic of each SNPs should be representative to the amount of variation they explain when there is no LD. Thus, based on this concept, SHREK targeted in “removing” the inflation of test statistic introduced through the LD between SNPs and the heritability can be calculated by adding the test statistic of all SNPs after “removing” the inflation.

Mathematically, the calculation of LDSC and SHREK were very different. LDSC take the sum of all R^2 within a 1cM region as the LD score and regress it against the test statistic to obtain the slope and intercept which represent the heritability and amount of confounding factors respectively. In their model, LDSC assume that each SNPs will explain the same portion of heritability

$$\text{Var}(\beta) = \frac{h^2}{M} \mathbf{I} \quad (2.38)$$

M = number of SNPs

β = vector containing per normalized genotype effect sizes

I = identity matrix

h^2 = heritability

As for SHREK, the whole LD matrix were used and inverted to decompose the LD from the test statistic. There were no assumption of the amount of heritability explained by each SNPs. However, SHREK does assumed that the null should be 1 and therefore SHREK cannot detect the amount of confounding factors.

2.3 Simulation

We implemented the heritability estimation in SHREK. To assess how well SHREK performs for heritability estimation in comparison to other current methods, we performed series of systematic simulation. In these simulations, performance of SHREK, GCTA(J Yang et al., 2011) and LDSC(B. K. Bulik-Sullivan et al., 2015) with and without the intercept estimation function (-no-intercept) were tested. Through simulation, we can obtain the sample distribution of the heritability estimate under different study designs (e.g. Quantitativat traits, Case-Control studies or extreme phenotype selection). Factors considered in our simulations were as follow:

2.3.1 Sample Size

The sample size was one of the most important parameter in determining the standard error of the heritability estimation. As the sample size increases, study will be more representative of the true population and therefore generate a more stable and accurate results.

Using simple text mining technique, we have calculated the average sample size for all GWAS recorded on the GWAS catalog(Welter et al., 2014) was around 7,200 samples. We argued that if the tools were able to preform well when the sample size was small, then they should have no problem handling larger data sets for the sample size only affect the standard error and should not bias the estimate. Thus, we only simulate 1,000 samples in our simulation to test the performance of the tools when a small sample size was provided.

2.3.2 Number of SNPs in Simulation

As technology progress, the number of SNPs on each array chip has increased significantly. We can now achieve a single base pair resolution using the next generation sequencing (NGS) technologies. However, in simulation, the number of SNPs is one of the biggest determinant of speed. The time required for simulation raised form 2 hours to 2 days when the number of SNPs increased from 3,000 to 50,000. Considering that one usually partition the whole genome into individual chromosomes during the heritability estimation, and that it was infeasible for us to simulate SNPs number similar to those encountered in real genotype chip, we compromise the number of SNPs in our simulation to 50,000 SNPs from chromosome 1.

2.3.3 Genetic Architecture

Of all the simulation parameter, the genetic architecture was arguably the most complicated parameter. It involves the LD pattern, the distribution of effect size, the number of causal SNPs, the maf of the causal SNPs and most importantly, the heritability of the trait (h^2).

Because we would like to cover most of the heritability spectrum, we will simulate traits with h^2 ranging from 0 to 0.9, with increment of 0.1 such that $h^2 \in \{0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9\}$. To simplify the condition, all “causal” variants were included in the simulation (e.g. perfect tagging). We also try to obtain realistic LD pattern by using HAPGEN2(Su, Marchini, and Donnelly, 2011) to simulate genotype based on the LD pattern of the 1000 genome Northern Europeans from Utah (CEU) samples.

First, we only consider situation of common SNPs and only simulate SNPs with $\text{maf} > 0.1$. In these simulation, we varies the number of causal SNPs (k) and the effect size distribution. We consider $k \in \{5, 10, 50, 100, 250, 500\}$ such that we can cover different disease spectrum (Oligogenic to Polygenic diseases). As for effect size distribution, we considered two conditions:

1. Equal Effect Size
2. Random Effect Size

The simplest situation was when all causal SNPs have the same effect size

$$\beta_s = \pm \sqrt{\frac{h^2}{k}} \quad (2.39)$$

The direction of effect should be randomly simulated. As for the random effect size scenario, we consider the effect size to be

$$\beta_r = \pm \sqrt{\frac{\gamma \times h^2}{\sum \gamma}} \quad (2.40)$$

with $\gamma \sim \exp(\lambda = 1)$ and a random direction of effect. Rationale behind the choice of exponential distribution was based on Orr (1998), which suggested that exponential distribution with $\lambda = 1$ may serve as a heuristic expectation the genetic architecture of adaptation.

Once the effect size was calculated, we can then randomly assign the effect size to k random SNPs with normalized genotype \mathbf{X} , the phenotype can then be calculated as

$$\begin{aligned} \epsilon_i &\sim N(0, \sqrt{\text{Var}(\mathbf{X}\boldsymbol{\beta}) \frac{1-h^2}{h^2}}) \\ \boldsymbol{\epsilon} &= (\epsilon_1, \epsilon_2, \dots, \epsilon_n)^t \\ \mathbf{y} &= \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\epsilon} \end{aligned} \quad (2.41)$$

For each batch of simulated samples, we calculate the estimated heritability using SHREK, GCTA, LDSC with intercept fixed at 1 and LDSC allowing for intercept estimation for each h^2 . In each iteration, the sample genotype was provided to GCTA for the calculation of genetic relationship matrix (GRM) whereas for SHREK and LDSC, 500 independent samples were simulated based on the 1000 genome project CEU samples (Project et al., 2012) to construct the LD matrix and calculate the LD score respectively. This was because both LDSC and SHREK were designed to work in situation where the raw genotype were not provided and the LD structure was usually obtained from the public data base instead. Therefore to provide a realistic simulation, an independent set of reference samples were provided for SHREK and LDSC.

The whole process were repeated 50 times with the same SNPs set, the same causal SNPs and the same effect size for each h^2 such than an empirical variance can be obtained. We then repeat the process 10 times on different SNPs sets, with different causal SNPs but

the same effect size for each h^2 . This should introduce a slight variation in the LD structure and should demonstrate the robustness of the programmes under different LD construct. To summarize, the simulation procedure follows:

1. Randomly select 50,000 SNPs with $\text{maf} > 0.1$ from chromosome 1
2. Randomly generate k effect size with $k \in \{5, 10, 50, 100, 250, 500\}$ following either eq. (2.39) or eq. (2.40)
3. Randomly assign the effect size to k SNPs
4. Simulate 1,000 samples using HAPGEN2 and calculate their phenotype according to eq. (2.41)
5. Perform heritability estimation using SHREK, LDSC and GCTA
6. Repeat step 4-5 50 times
7. Repeat step 1-6 10 times

Extreme Effect Size

Another condition we were interested in was the performance of the tools when there is a small amount of SNPs that explain a large portion of effect e.g. 50%. Similarly, we only consider 1,000 samples, with 50,000 common SNPs($\text{maf} > 0.1$). We hypothesize that under the polygenic model, such extreme distribution in effect size should have much larger effect when compared to that in oligogenic condition. Thus we only consider the polygenic condition where the number of causal SNP (k) were limited to 100 or 250.

We simulate m SNPs accounting for 50% of all the effect where $m \in \{1, 5, 10\}$. The effect size was then calculated as

$$\beta_{eL} = \pm \sqrt{\frac{0.5h^2}{m}} \beta_{eS} = \pm \sqrt{\frac{0.5h^2}{k-m}} \beta = \{\beta_{eL}, \beta_{eS}\} \quad (2.42)$$

the effect size were then randomly assigned to k causal SNPs and phenotype was calculated as in eq. (2.41). The simulation procedure then becomes

1. Randomly select 50,000 SNPs with $\text{maf} > 0.1$ from chromosome 1
2. Randomly generate k effect size with $k \in \{100, 250\}$ and m extreme effect, following eq. (2.42) where $m \in 1, 5, 10$

3. Randomly assign the effect size to k SNPs
4. Simulate 1,000 samples using HAPGEN2 and calculate their phenotype according to eq. (2.41)
5. Perform heritability estimation using SHREK, LDSC and GCTA
6. Repeat step 4-5 50 times
7. Repeat step 1-6 10 times

Rare SNPs

Based on the “common disease - rare variant” hypothesis, a complex disease might still be affected by number of rare genetic variants. With the advancement of technology, there are now genotype arrays such as exome chip which aim to detect some of the rare variants. Thus we were interested in testing the performance of the tools under such extreme scenario.

Here, we will consider the situation where of the k causal SNPs, r of them were rare (e.g. $\text{maf} < 0.05$). Instead of simulating only common SNPs, we simulate 50,000 SNPs with $\text{maf} > 0.01$. Then we simulate k effect size using equation eq. (2.39). However, instead of randomly distributing the effect size to k random SNPs, we distribute the effect size to r rare SNPs ($\text{maf} < 0.05$) and $k - r$ common SNPs ($\text{maf} > 0.05$) with $r \in \{5, 10\}$. Again, the phenotype was calculated using eq. (2.41).

2.3.4 Case Control Studies

The simulation of case control studies was very much like that of the simulation of quantitative trait. However, there were two additional parameters to consider: the population prevalence and the observed prevalence. These parameters allow us to simulate the samples under a liability model, therefore simulating the case control studies.

Although there were only two additional parameter, the computational challenge for the simulation of case control was significantly bigger than that for the simulation of quantitative trait. Take for example, if one like to simulate a trait with population prevalence of p and observed prevalence of q and would like to have n cases in total, one will have to simulate $\min(\frac{n}{p}, \frac{n}{q})$ samples. Considering the scenario where the observed prevalence is 50%, the population prevalence is 1% and 1,000 cases,a minimum of 100,000 samples will be

required. If each samples have 50,000 SNPs, then one will need to process a minimum of 40GB of data per iteration.

Given limited computer resources, we simulate 1,000 cases with $q = 0.5$ and varies $p \in \{0.5, 0.1\}$ as a prove of concept simulation. We argue that although we only consider a limited combination of prevalence, the result should be generalize to other combination of prevalence.

2.3.5 Extreme Phenotype Selection

2.3.6 Case Control Studies

Similar to quantitative trait simulation, the sample size, the number of SNPs, the number of causal SNPs and the true heritability of the trait are important parameters to consider during simulation. On top of that, there are a few more parameters one must consider during the simulation of case control studies such as the population prevalence of the trait and the observed prevalence of the study.

To simulate cases and controls, we will need to simulate the liability distribution by taking into account of the prevalence. So for example, if one like to simulate a trait with population prevalence of p and observed prevalence of q and would like to have n cases in total, one will have to simulate $\min(\frac{n+pn}{p}, \frac{n+qn}{q})$ samples. It is therefore challenging for one to simulate scenario with a small p or q values.

In this study, we fixed $q = 0.5$ and varies $p \in \{0.5, 0.1\}$. Although disease such as schizophrenia (SCZ) can have a prevalence $\approx 1\%$, the required sample numbers become infeasible for large scale simulation where a minimum of 101,000 samples will be required if we wish to obtain 1,000 cases. Despite our wish to simulate conditions with small prevalence, the limitation of computation power simply forbade us to undergo such simulation.

Once the liability distribution were simulated, cases can be drawn from samples with a liability higher than the liability threshold. The liability threshold was calculated as the value $> 1-p$ of all values under the standard normal distribution using the *qnorm* function in R. Samples with a liability lower than the liability threshold were then considered as control samples. 1,000 cases and 1,000 controls were then randomly drawn from the corresponding population of samples.

To summarize,

1. Randomly select 50,000 SNPs from chromosome 1
2. Randomly generate k effect size following ?? where $k \in \{10, 50, 100, 1000\}$
3. Randomly assign the effect size to k SNPs where SNPs with small maf will get a large effect size.
4. Simulate $\frac{n+qn}{q}$ samples using HAPGEN2 where $q \in \{0.5, 0.1\}$
5. Simulate sample phenotype according to the liability threshold where 1,000 cases and 1,000 controls were obtained
6. Perform heritability estimation using SHREK, LDSC and GCTA
7. Repeat step 4-6 50 times
8. Repeat step 1-7 50 times

2.3.7 Extreme Phenotype Selections

The simulation of extreme phenotype selection is very much like the combination of quantitative trait simulation. The simplest way to simulate the extreme phenotype selection is to first simulation N samples, then from this population of samples, select n samples from both end of the population and use them to perform association and heritability estimation.

Due to the similarity in nature with the simulation of quantitative traits and case control studies, we limit the number of causal SNPs simulated as 100. We also limit our simulation to select samples with phenotype on the top and bottom 10% of the population.

However, it was noted that both GCTA and LDSC did not implement heritability estimation under extreme phenotype selection. To perform a fair comparison with SHREK, we calculate the adjustment factor $\frac{\text{Var}(\text{Phenotype before selection})}{\text{Var}(\text{Phenotype after selection})}$ to the estimation from GCTA and LDSC.

Therefore, to summarize,

1. Randomly select 50,000 SNPs from chromosome 1
 2. Randomly generate 100 effect size following ??
 3. Randomly assign the effect size to k SNPs where SNPs with small maf will get a large effect size.
-

4. Simulate 10,000 samples using HAPGEN2 and calculate their phenotype according to eq. (2.41)
5. Select the top and bottom 10% of samples from the 10,000 samples.
6. Perform heritability estimation using SHREK, LDSC and GCTA
7. Manually apply the adjustment factor to estimation of GCTA and LDSC
8. Repeat step 4-7 50 times
9. Repeat step 1-8 50 times

2.4 Result

The heritability estimation were implemented in SHREK and is available on <https://github.com/choishingwan/shrek>.

2.5 Discussion

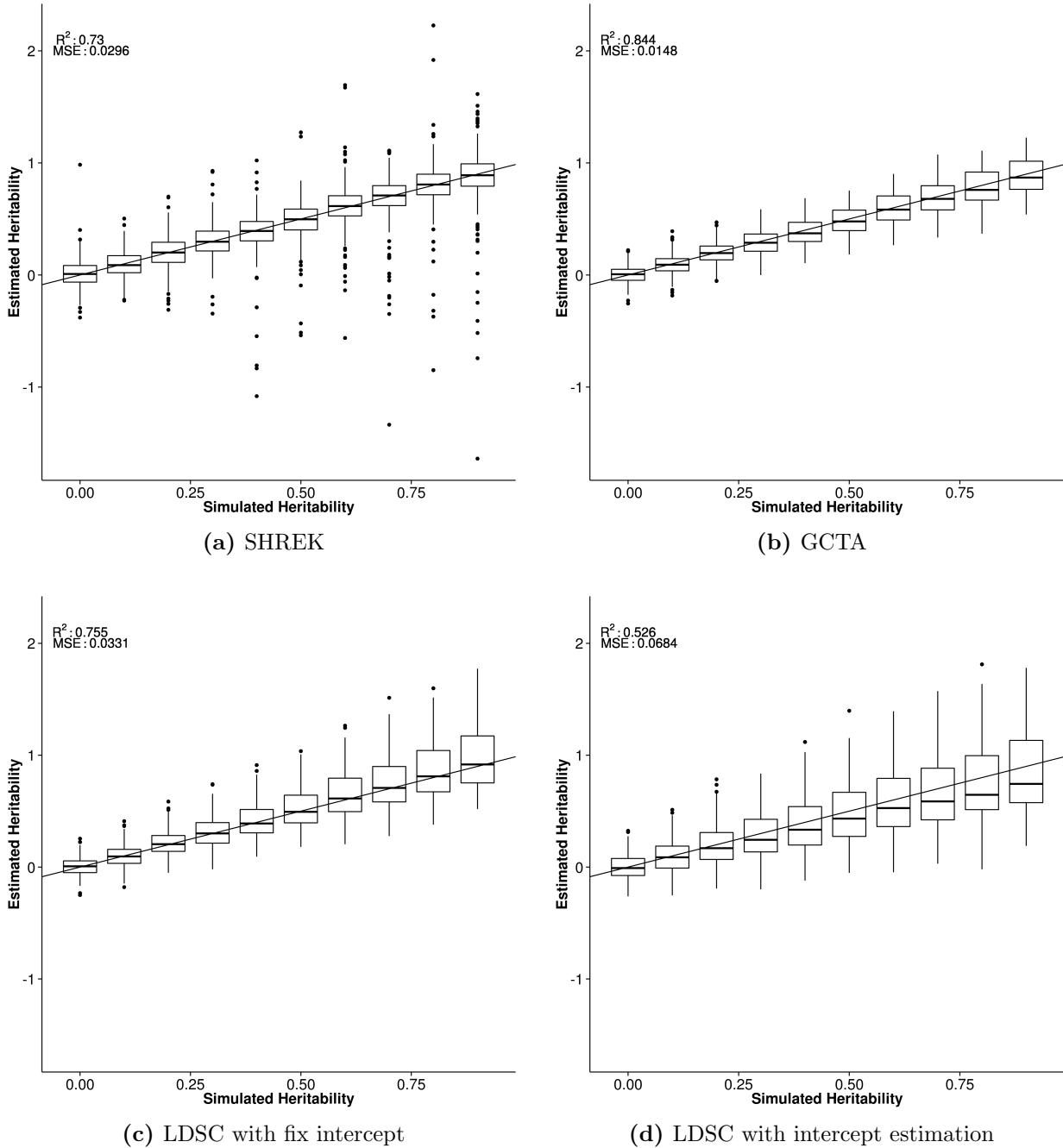
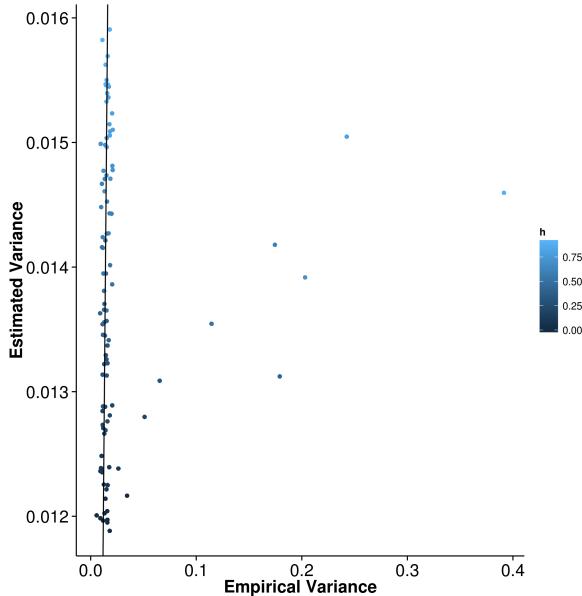
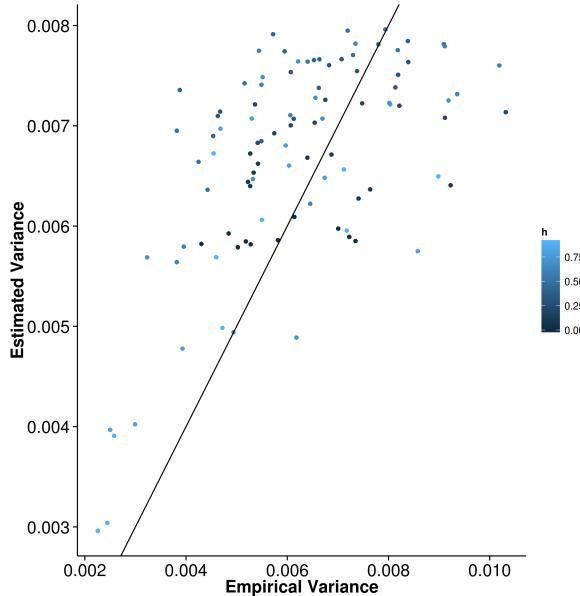


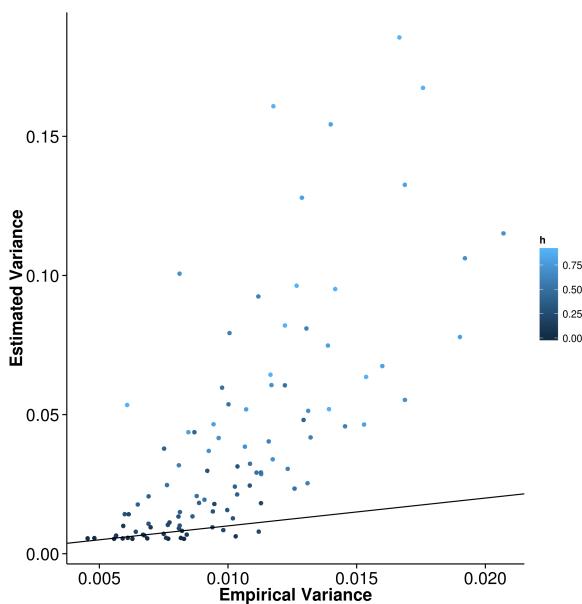
Figure 2.2: Simulation of Quantitative Traits with 50k SNPs and 10 causal variants with same effect size. It was observed that of all the tools, SHREK performed best in such scenario



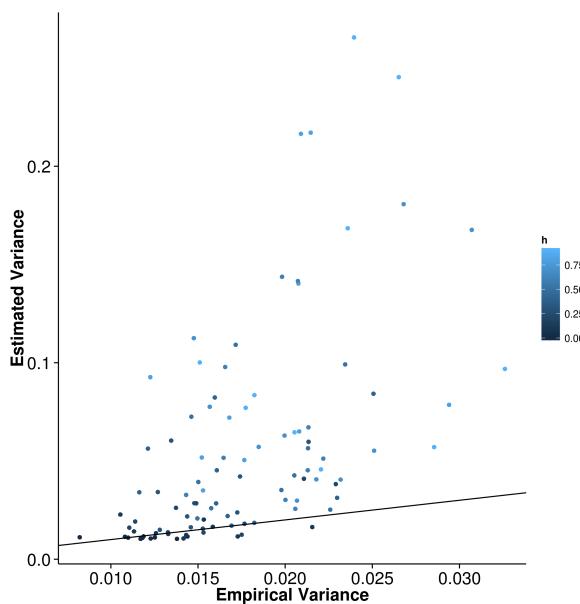
(a) SHREK



(b) GCTA



(c) LDSC with fix intercept



(d) LDSC with intercept estimation

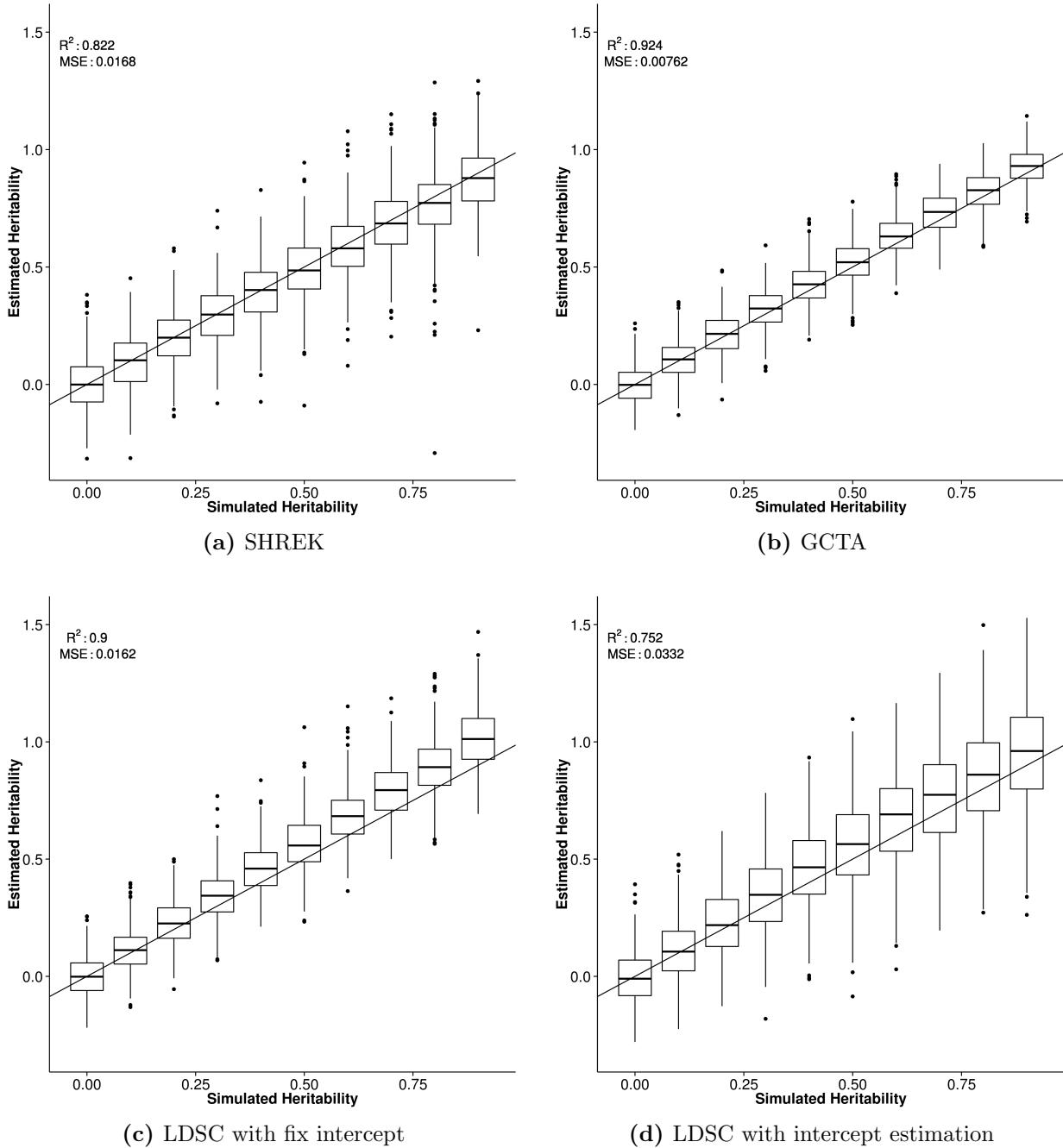
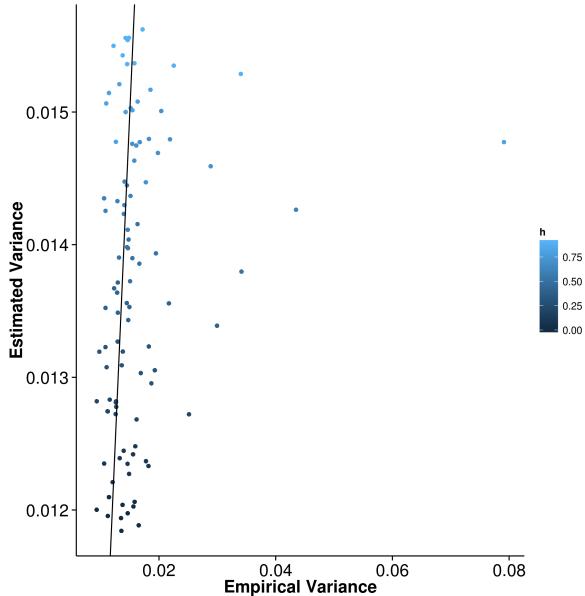
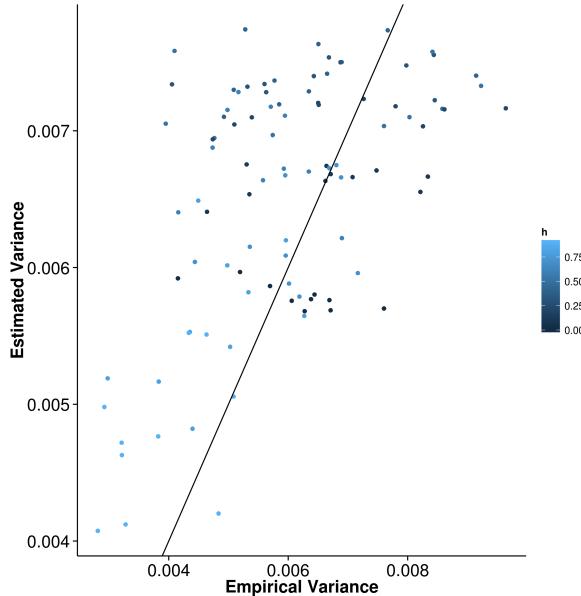


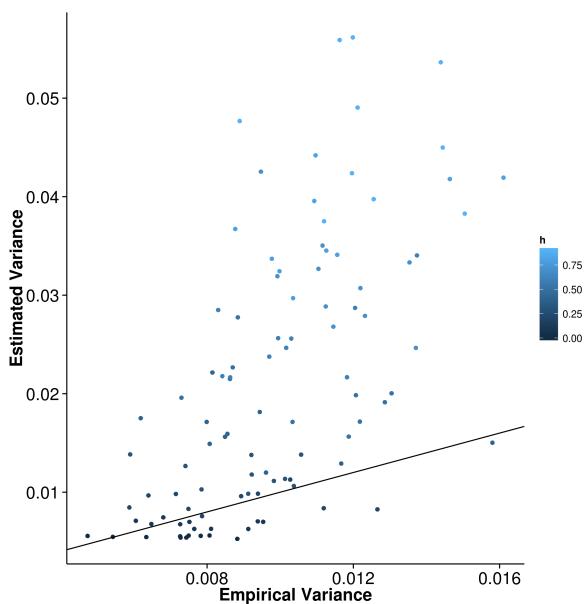
Figure 2.3: Simulation of Quantitative Traits with 50k SNPs and 50 causal variants with same effect size.



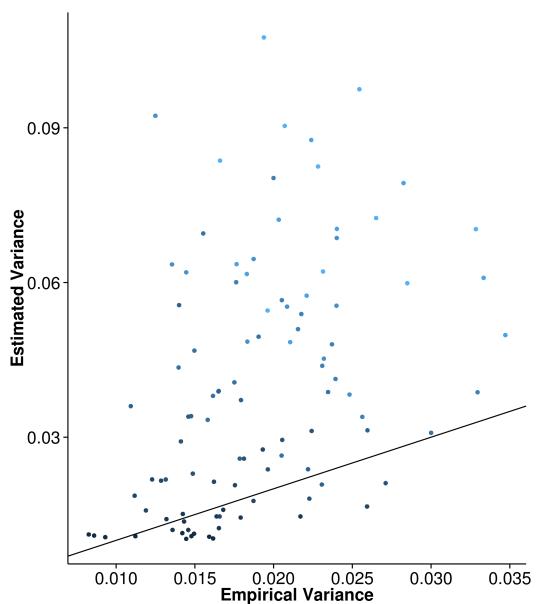
(a) SHREK



(b) GCTA



(c) LDSC with fix intercept



(d) LDSC with intercept estimation

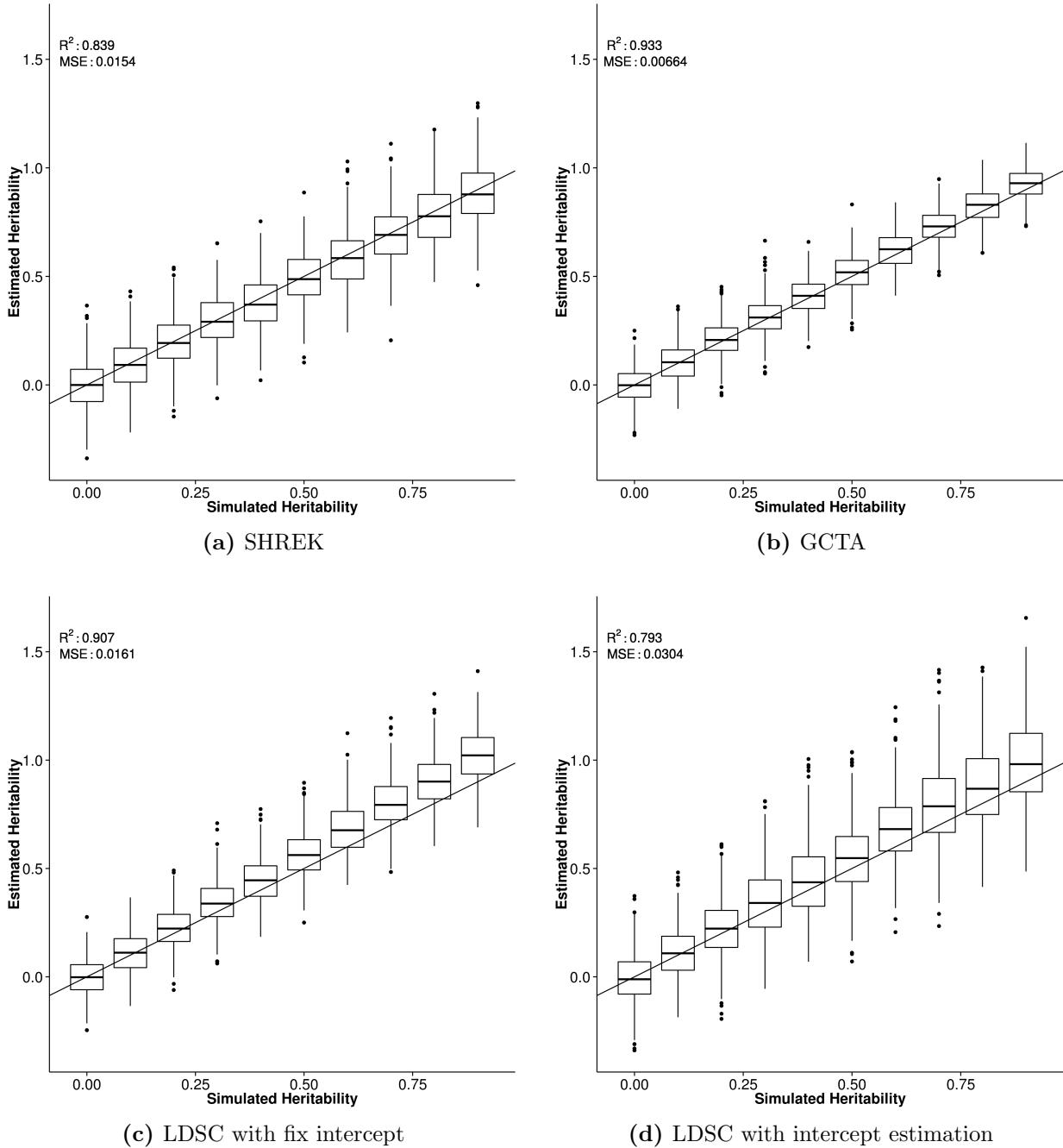
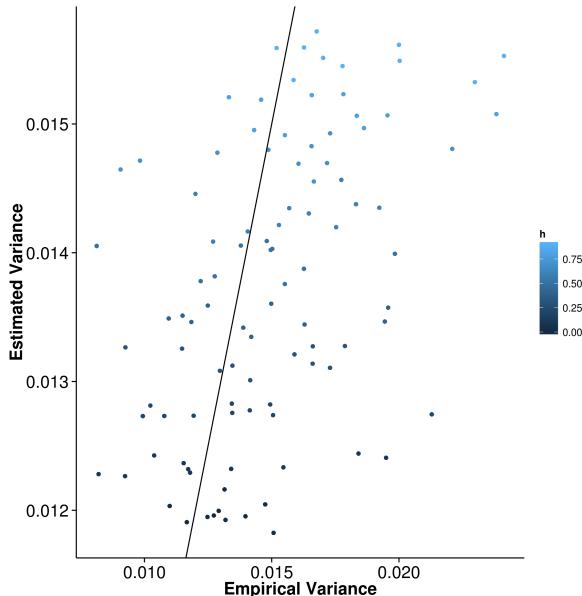
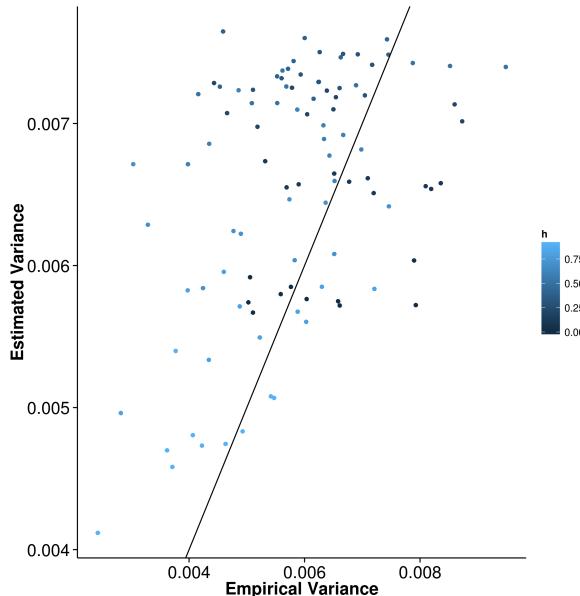


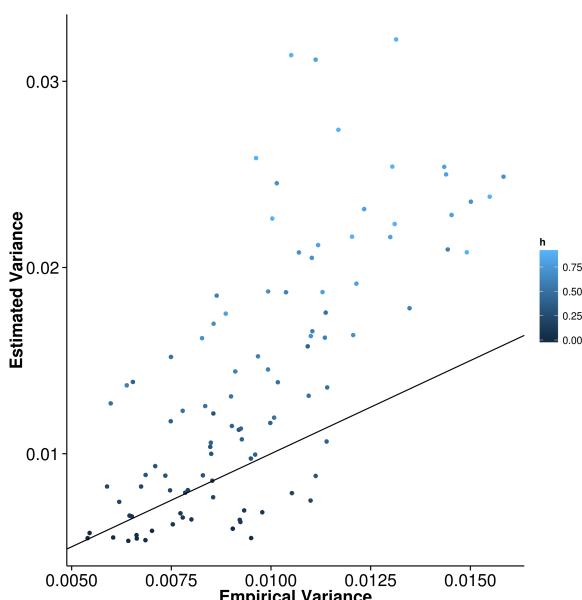
Figure 2.4: Simulation of Quantitative Traits with 50k SNPs and 100 causal variants with same effect size.



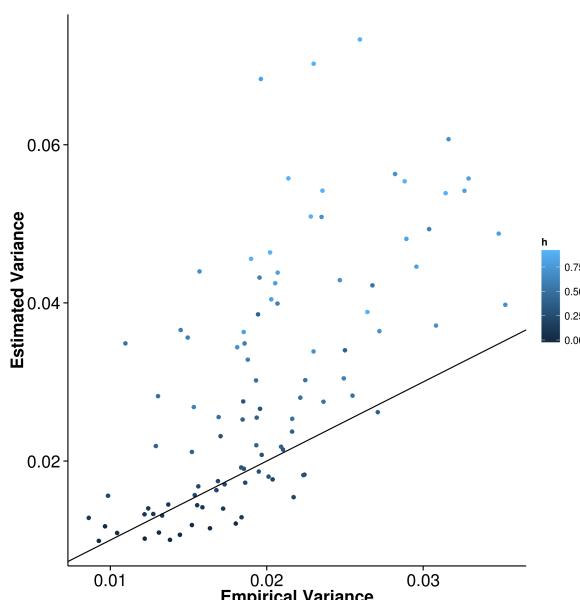
(a) SHREK



(b) GCTA



(c) LDSC with fix intercept



(d) LDSC with intercept estimation

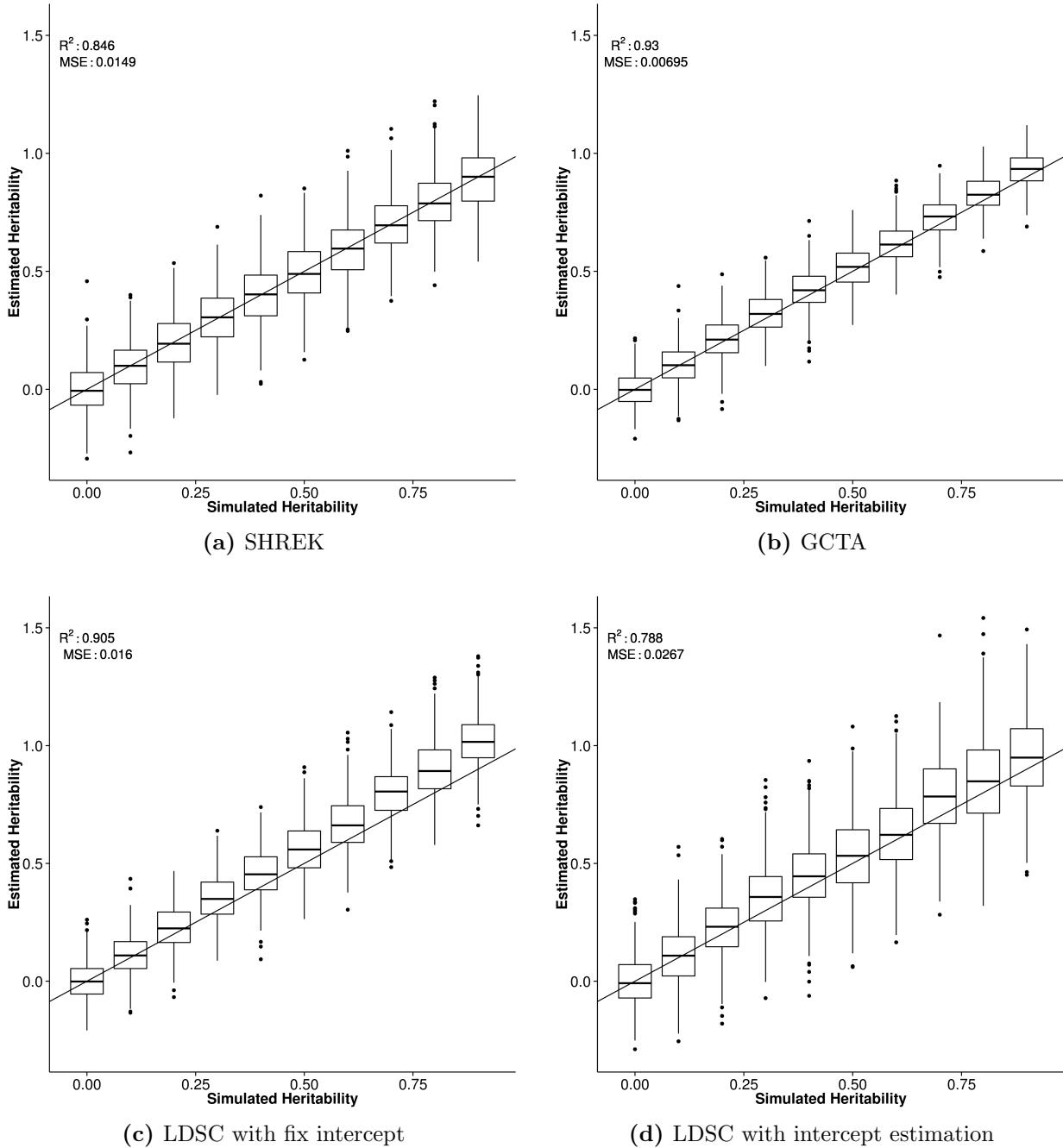
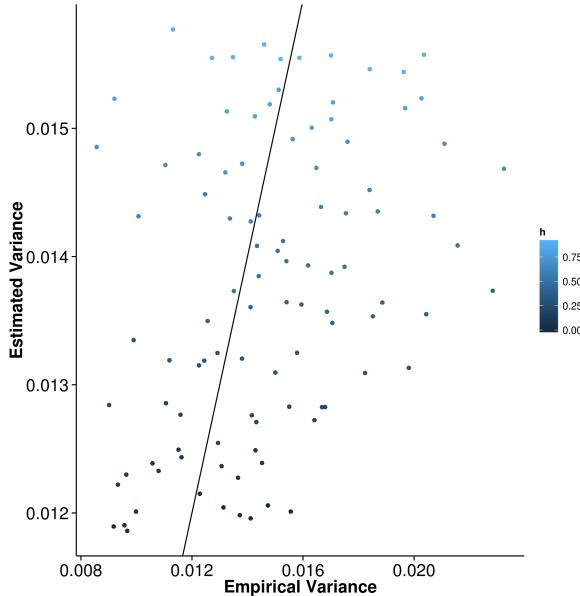
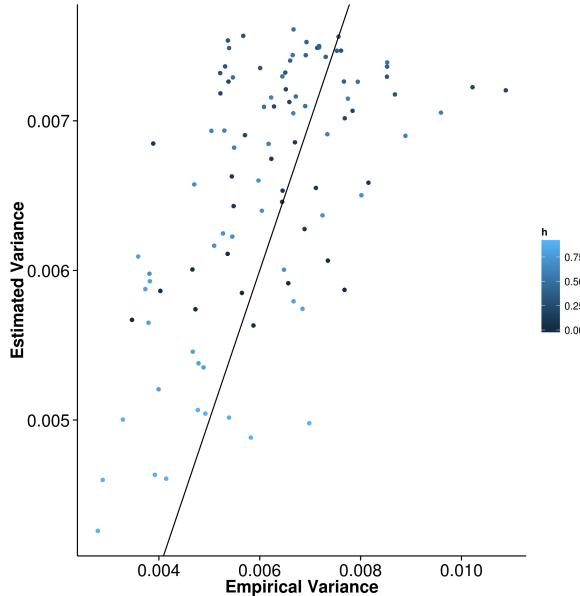


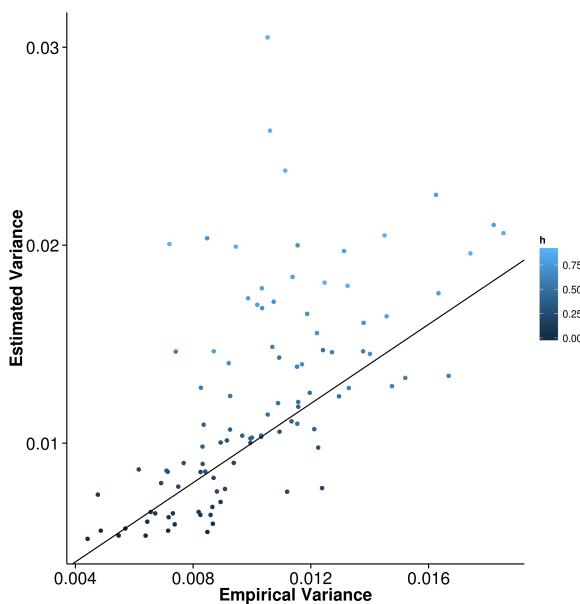
Figure 2.5: Simulation of Quantitative Traits with 50k SNPs and 250 causal variants with same effect size.



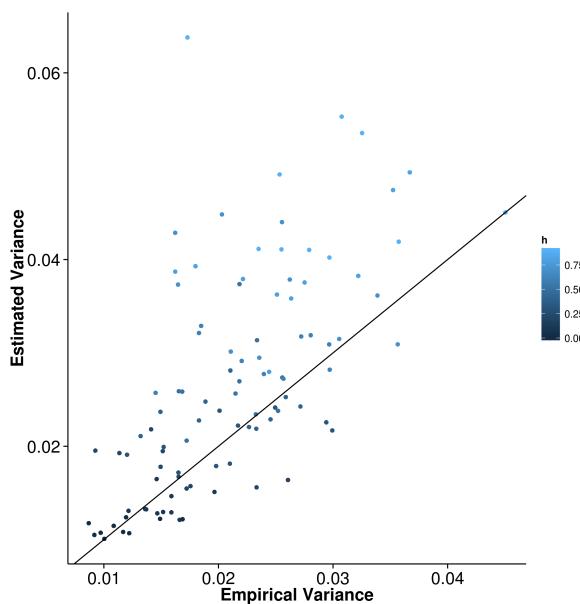
(a) SHREK



(b) GCTA



(c) LDSC with fix intercept



(d) LDSC with intercept estimation

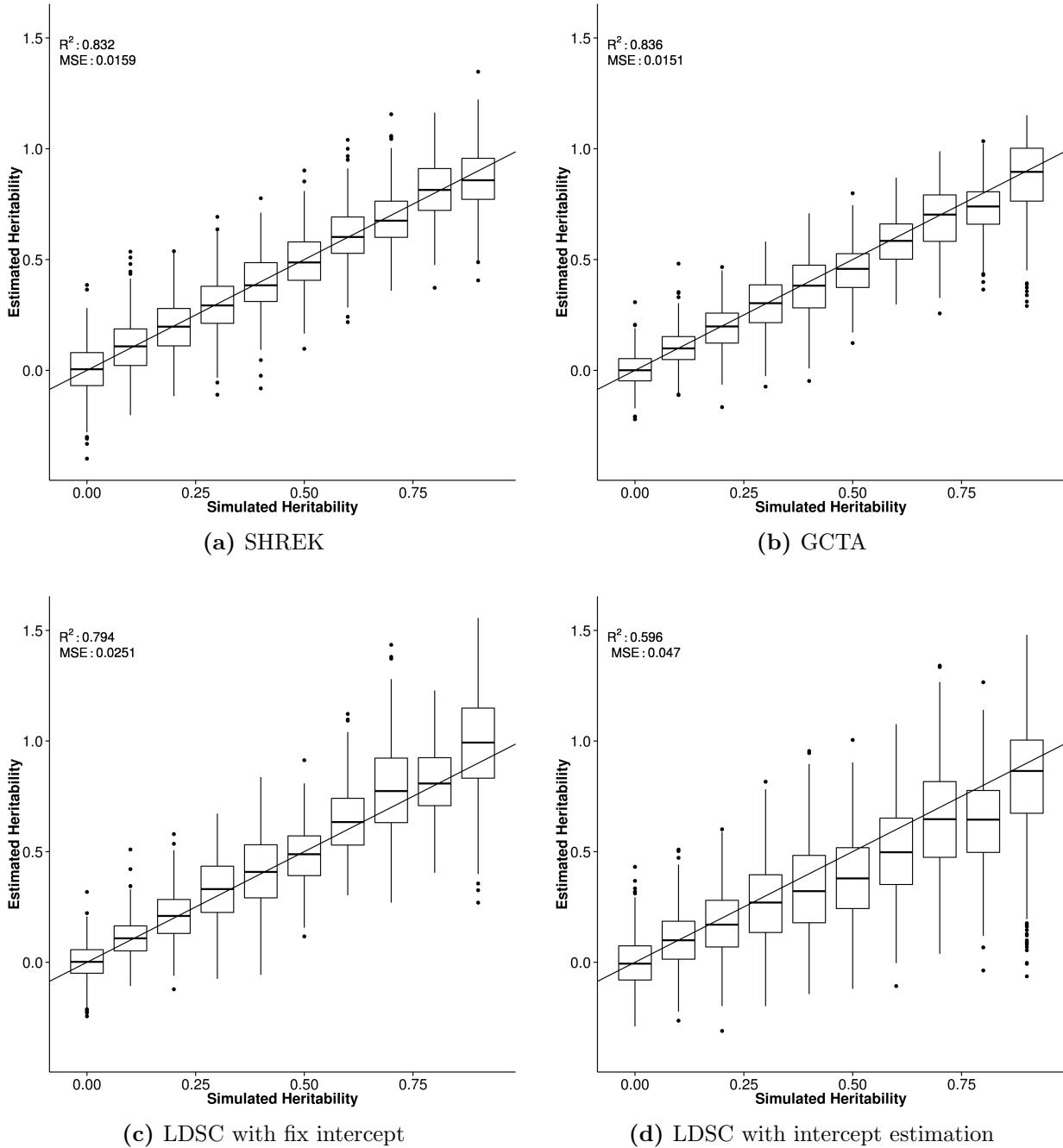
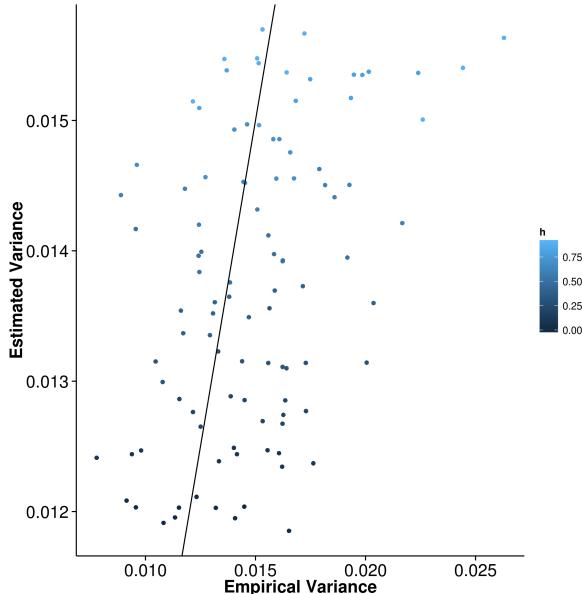
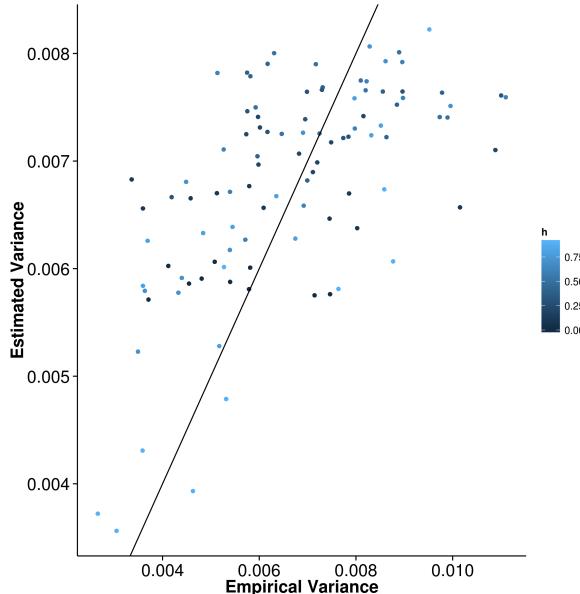


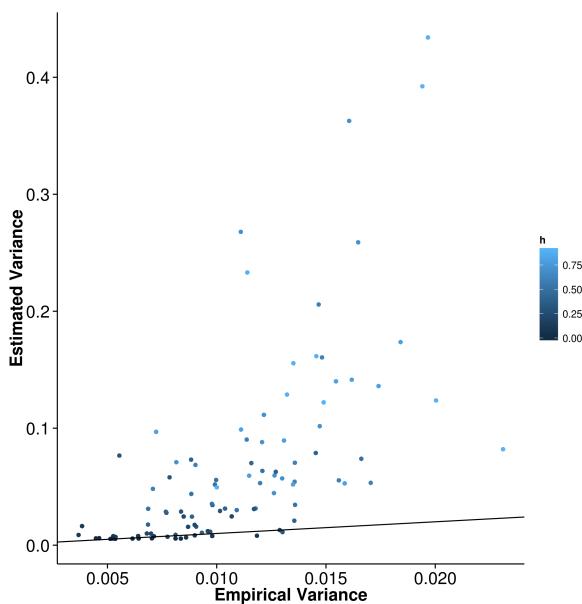
Figure 2.6: Simulation of Quantitative Traits with 50k SNPs and 10 causal variants with random effect size.



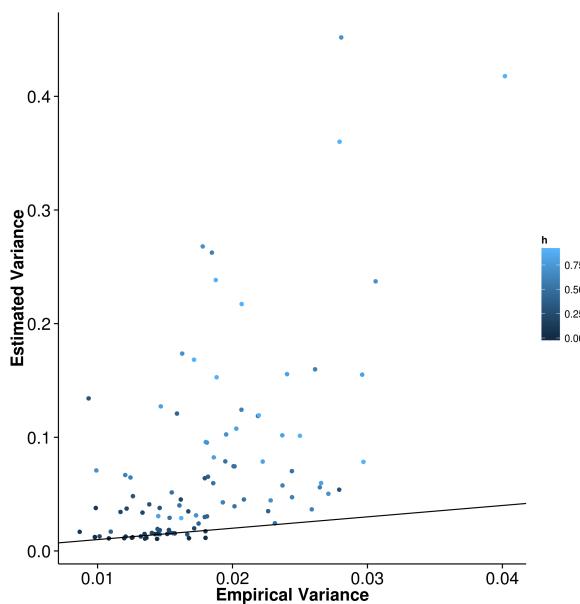
(a) SHREK



(b) GCTA



(c) LDSC with fix intercept



(d) LDSC with intercept estimation

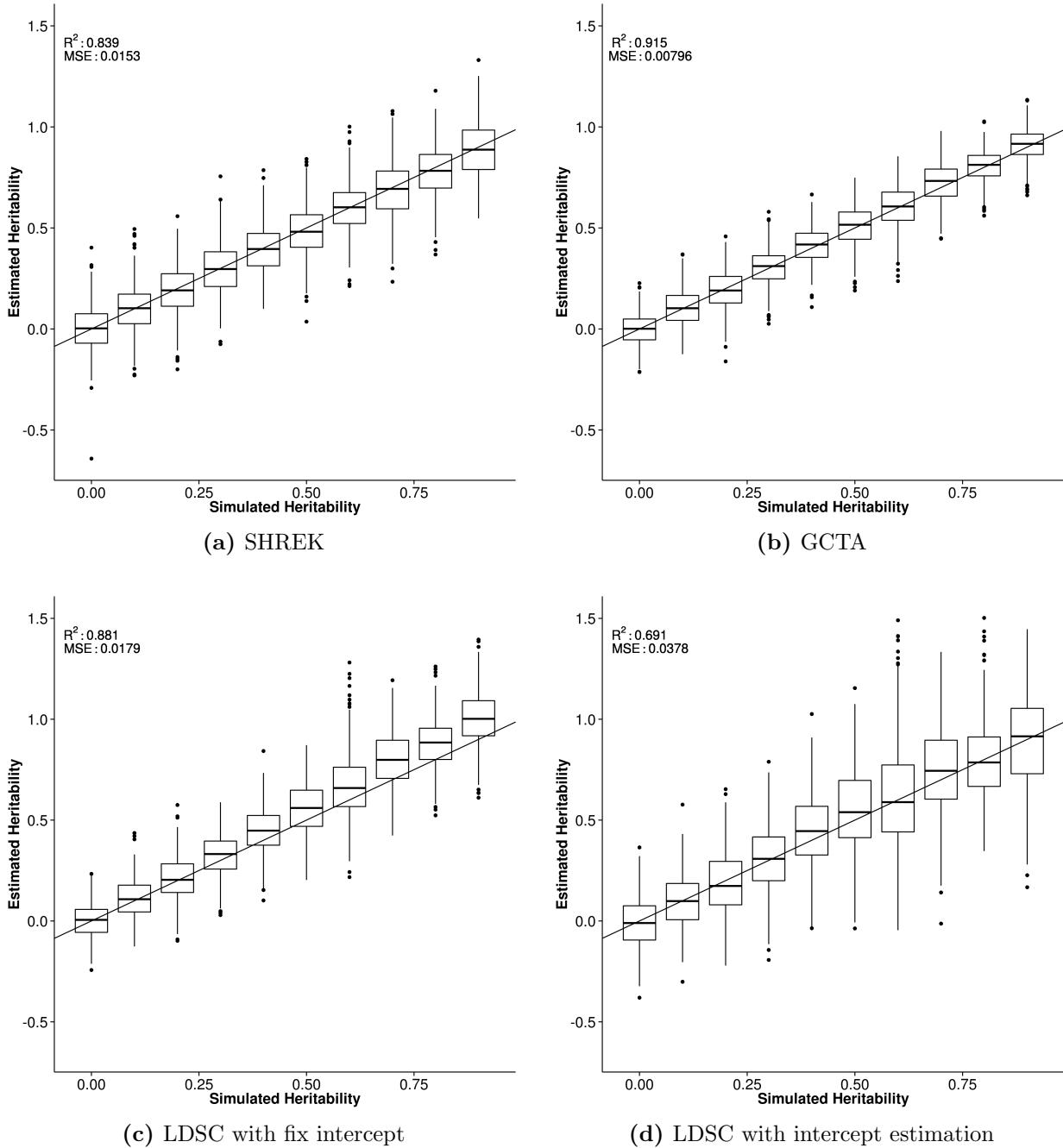
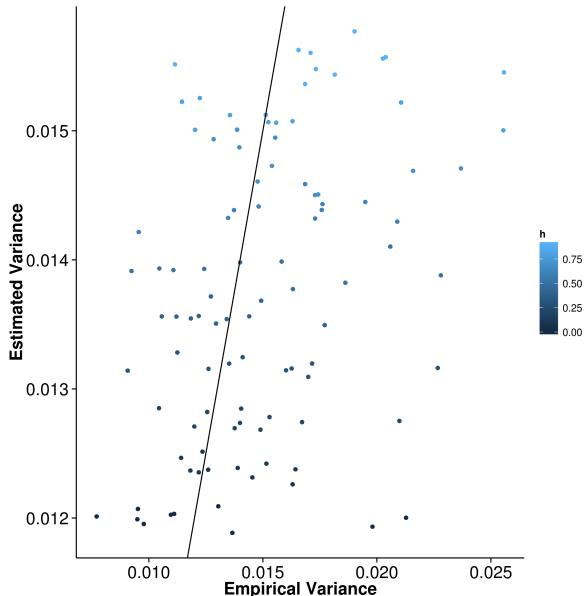
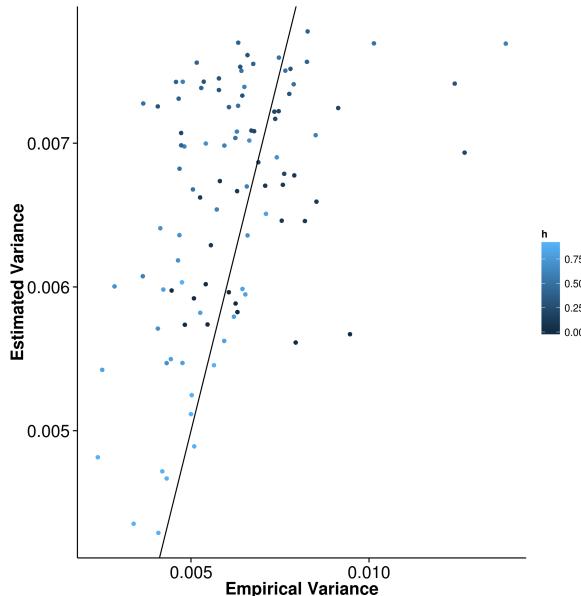


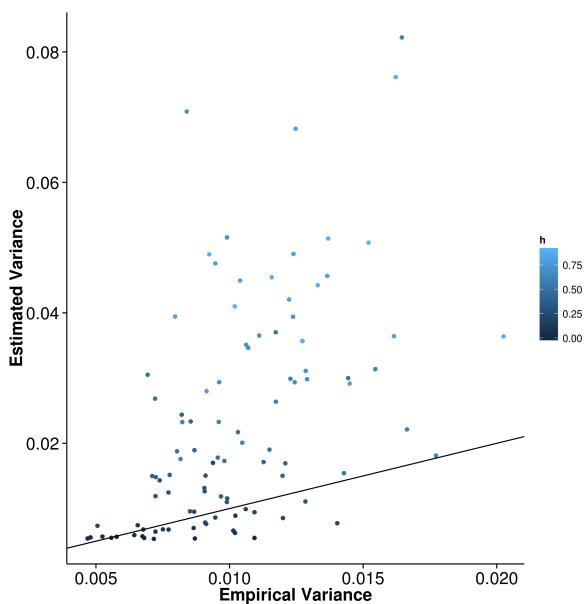
Figure 2.7: Simulation of Quantitative Traits with 50k SNPs and 50 causal variants with random effect size.



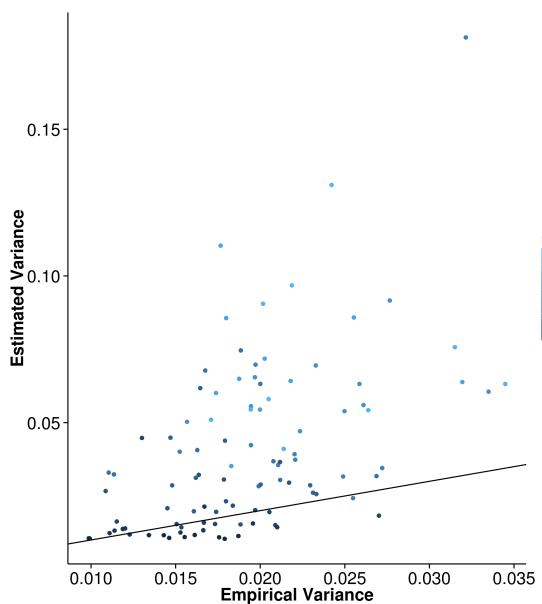
(a) SHREK



(b) GCTA



(c) LDSC with fix intercept



(d) LDSC with intercept estimation

Chapter 3

Heritability of Schizophrenia

3.1 Introduction

Apply Heritability estimation to the schizophrenia data. The genetic correlation and partitioning of heritability No one worked on linking schizophrenia with brain development directly?

3.2 Heritability Estimation

This will be a very simple section, focused on how to perform the heritability estimation on schizophrenia (SCZ). Should also tokenize the heritability into subcategories (e.g. immune, neuron, etc)

3.2.1 Methodology

3.2.2 Result

3.3 Brain development and Schizophrenia

Here we will perform the WGCNA and brain development network. Seeing how the whether if any brain development network were enriched with SNPs that explain the variance of

phenotype

3.3.1 Methodology

Sample Quality Controls

We obtain the developmental transcriptome data from BrainSpan (<http://www.brainspan.org/>). A total of 56 samples with different age were provided by BrainSpan with an average of 2.2 samples per age.

Studies suggested Hippocampus(Velakoulis et al., 2006; Nugent et al., 2007), Amygdala and Striatum(Simpson, Kellendonk, and Kandel, 2010) are brain regions involved in the etiology of schizophrenia. Therefore, we focus on building the gene co-expression network of hippocampus, amygdala and striatum in this study. It is worth noting that the Pre-frontal Cortex is also important for schizophrenia. However, as there isn't a well defined pre-frontal cortex samples from BrainSpan, we did not include the pre-frontal cortex in the current study. RNA Sequencing data of the brain regions were obtained from BrainSpan and undergo a series of quality control before the construction of the network.

For each sample age, when there are more than one samples, we select the sample with a dissection score ≥ 3 and an RNA integrity number (RIN) ≥ 7 . As some developmental stage only got 1 sample passing the quality check, we limit each developmental stage to have a maximum of 1 sample such that the final network will not be driven by a particular developmental stage. If multiple samples passed through the quality check threshold, we will prefer sample with higher dissection score. Shall multiple samples have the same dissection score, we will select the one with the highest RIN. And if the samples have the same dissection score and RIN value, we will randomly select one for the network construction.

After performing the quality control, a total of 16, 18 and 15 samples were selected for hippocampus, amygdala and striatum respectively. The sample age ranged from Gestation Day (GD)8 to 23 years old representing the fetal developmental stage till the age of onset of schizophrenia.

Normalization of data

The RNA Sequencing data were represented as Reads Per Kilobase per Million mapped reads (RPKM) values. Genes with a low RPKM can usually be a result from technical or biological

3.3. BRAIN DEVELOPMENT

noise(Hart et al., 2013). To reduce noise in the final model, genes with a mean RPKM < 1 in all samples were discarded. The RPKM were then log transformed as instructed by the manual of Weighted Gene Co-expression Network Analysis (WGCNA)(Langfelder and S Horvath, 2008).

As there are insufficient samples for the construction of gene co-expression network for individual sample age, we try to construct networks with genes co-expressed through all sample stage. This is achieved by taking the standardized \log_2 RPKM across sample age such that all genes has a mean of 0 and standard deviation of 1.

At the end, there were 17,168 genes, 17,038 genes and 17,166 genes passing through the quality threshold and were used for the construction of co-expression network in hippocampus, amygdala and striatum respectively.

Network Construction

WGCNA (ver 1.47) were used for the construction of gene co-expression network(Langfelder and S Horvath, 2008). The *blockwiseModules* function, using Biweight Midcorrelation for the construction of correlation matrix and a restriction of minimum network size of 30. For the construction of gene co-expression networks in hippocampus, the soft-power threshold were set to 15 where it is the first threshold value which has $R^2 > 0.8$ (0.817) and the R^2 is saturated(Zhang and Steve Horvath, 2005).As for striatum, the soft-power threshold were set to 20. Again, this is the first threshold value with $R^2 > 0.8$ (0.879) and where the R^2 is saturated.

On the other hand, for amygdala, soft-power threshold were set to 9 which is the first threshold for R^2 to reach saturation. However, with a soft-power threshold of 9, the R^2 were only 0.776, which is lower than the recommended 0.8 threshold. The reason behind this decision was that the first soft-power threshold to have $R^2 > 0.8$ is 30. Under this threshold, the mean connectivity of the resulting networks will be around 23.6 with a median connectivity of 2.51. Such level of connectivity will likely yield networks that are too small to useful. If one would like to satisfy both requirement of threshold selection, a threshold > 30 are likely required and any networks constructed will likely to be small. As a result of that, we select threshold of 9 where networks with reasonable size can be constructed.

Expression correlation with Age

The co-expression network constructed with the standardized gene expression value will contain genes that co-express in all sample age. However, this does not necessarily suggest the expression of these genes are correlated with the sample age. To identify gene co-expression networks with expressions correlated with the sample age, we performed a correlation analysis between the module eigen-genes and the sample age. Network eigen-genes were calculated as the first Principle Component (PC) of expressions of the genes within individual networks using the *moduleEigengenes* function from WGCNA. Age were represented as month from conception such that 8 post-conception week will be represented as 2; 4 months will be represented as 10 and 12 years will be represented as 154 etc. Finally, correlation between age and network eigen-gene expression were calculated pearson correlation.

Functional Annotation

Gene Ontology (GO) based enrichment analysis of the significant module was performed using GOrilla(Eden et al., 2009). Genes within the networks were provided as the target gene lists and all the genes passed quality controls were used as the background gene list. As GO terms tends to be redundant and overlaps with each other, it will aid the interpretation of GO results based by clustering and reducing the GO terms based on their similarity. Thus, GO enrichment results were summarized by REViGO(Supek et al., 2011) and significant representative GO terms were obtained.

Associate Co-expression network with PGC schizophrenia data

The co-expression networks were built from normal samples and should not be representative of the brain expression pattern in schizophrenia patients. It is however interesting to see if the co-expression networks were disrupted in schizophrenia patient. To test whether if the gene co-expression networks contain genes that are jointly associated with schizophrenia, we first use Multi-marker Analysis of GenoMic Annotation (MAGMA)(Leeuw et al., 2015)(version v1.03) to compute the gene-base p-value from the SNP wise p-value obtained from PGC. Gene-set enrichment analysis were then performed on networks that were significantly correlated with developmental age. As we were only interested in whether if the genes within the networks were jointly associated with schizophrenia, we only focus on the result of the self-contained gene set analysis and ignore the result from competitive analysis.

Partitioning of Heritability

3.3.2 Result

Co-Expression Network

A total of 35 networks were constructed based on the hippocampus samples with a mean network size of 421.6. On the other hand, 28 networks were constructed for amygdala with mean network size of 591.86. Finally, 25 networks with mean size of 494.52 were constructed from the striatum samples.

Of the all the networks constructed, only one network from hippocampus(table 3.1a) and three networks from amygdala(table 3.1b) were significantly correlated with sample age after bonferroni correction threshold (p-value < 0.00143 for hippocampus, p-value < 0.00179 for amygdala and p-value < 0.002 for striatum) .

By plotting the mean expression of each network against the sample age, one can inspect how the dynamic of the network changes across different developmental stage. Thus, mean expression of all the genes within the significant networks were calculated for all amygdala (n=33) and hippocampus (n=32) samples from BrainSpan. The mean RPKM values were then \log_2 transformed and plot against the sample age where a line of bests fit was calculated using the *stat_smooth* with the loess function from R package *ggplot2*(version 1.0.1). (fig. 3.1).

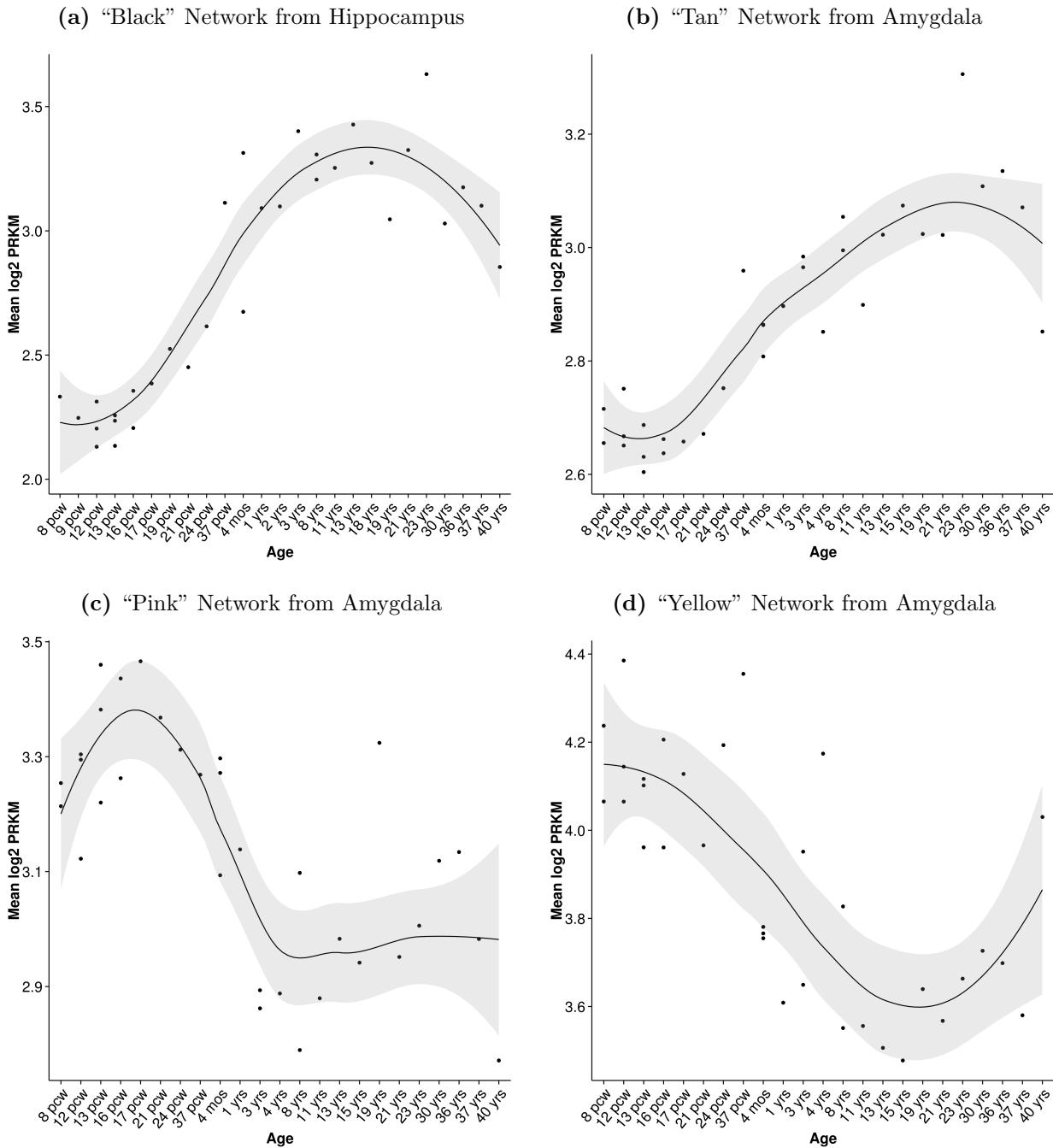
The expression pattern observed were intriguing where there both the “black” (fig. 3.1a) and “tan” (fig. 3.1b) networks have mean gene expression level increase as development progress and reaches its peak at around late adolescence ($\approx 18 - 21$), concurring with the onset age of schizophrenia. Similarly, an inverse pattern were observed with the “yellow” network where its mean expression was highest during fetal development and drop steadily to its lowest around late adolescence and increase again afterwards(fig. 3.1d).

The expression pattern of the “black” and “tan” networks are of particular interest as they follow the inverted “U” shape trajectory of the grey matter volumn observed in previous studies(Gogtay et al., 2011), suggest that they might have a role in mediating brain development.

Table 3.1: Correlation of sample age with the module eigen gene. Module eigen-gene was defined as the first PC of genes within the module. After correcting for multiple testing, only the black module was considered as significantly correlated with the sample age.

(a) Hippocampus			(b) Amygdala		
	Correlation	Pvalue		Correlation	P-value
black	0.804653	0.000171	tan	0.849999	7.96×10^{-6}
blue	-0.61648	0.010981	yellow	-0.757	2.76×10^{-4}
red	-0.60207	0.013595	pink	-0.68541	1.69×10^{-3}
darkred	-0.59137	0.015833	greenyellow	-0.67831	1.97×10^{-3}
greenyellow	-0.56995	0.021168	red	-0.64532	3.83×10^{-3}
yellow	0.567828	0.021763	turquoise	-0.59771	8.80×10^{-3}
darkgrey	-0.55246	0.026474	lightyellow	-0.56347	0.0149
saddlebrown	-0.52983	0.034783	brown	0.548516	0.0184
turquoise	-0.51371	0.041809	darkgreen	-0.46366	0.0526
purple	-0.46788	0.067606	blue	-0.4604	0.0545
darkolivegreen	-0.41272	0.112122	purple	-0.44182	0.0664
sienna3	-0.39535	0.129604	darkgrey	-0.39065	0.109
darkturquoise	0.386541	0.139154	orange	-0.36966	0.131
darkorange	0.384966	0.140912	white	0.28737	0.248
darkmagenta	0.375586	0.151688	darkred	0.283247	0.255
brown	0.366095	0.163144	black	0.271383	0.276
tan	-0.36522	0.164229	salmon	-0.24203	0.333
pink	0.348979	0.18524	skyblue	0.207071	0.410
magenta	-0.32559	0.218473	cyan	0.18778	0.456
midnightblue	-0.29168	0.273014	lightgreen	0.166495	0.509
lightgreen	0.289921	0.276056	grey60	0.15156	0.548
paleturquoise	-0.28045	0.29276	midnightblue	0.136078	0.590
white	0.27727	0.29849	magenta	-0.13459	0.594
orange	0.19607	0.466754	darkturquoise	0.129954	0.607
steelblue	0.17355	0.520357	lightcyan	0.090241	0.722
skyblue	0.145869	0.589857	darkorange	-0.05166	0.839
lightyellow	-0.11665	0.667028	green	-0.04745	0.852
green	-0.09882	0.715786	royalblue	0.020456	0.936
violet	-0.08757	0.747076			
lightcyan	-0.0656	0.809257			
cyan	-0.06441	0.812661			
darkgreen	-0.03914	0.885582			
salmon	0.038727	0.886769			
royalblue	-0.03785	0.889314			
grey60	0.03119	0.908709			

Figure 3.1: Mean Gene Expression across developmental age. Mean RPKM values of genes in the significant modules were plotted with respect to the sample age. A loess smoothing curve was also plotted.



Functional Annotation

Upon performing the GO enrichment analysis, a total of 16 GO terms were enriched in the “black” hippocampus network, 4 in the “tan” amygdala network and 45 in the “yellow” amygdala network. No GO term was enriched in the “pink” amygdala network.

The enriched GO terms of the “yellow” amygdala network were mainly related to translation and transcription and were not specific to brain function or development(??). On the contrary, the GO terms enriched in the “black” hippocampus network were highly relevant to brain function and development (table 3.2)(e.g. “central nervous system development” and “glutamate metabolic process”) and the “tan” amygdala network were also related to ammonium ion metabolism (table 3.3) which is vita for glutamine synthesis from glutamate(Liaw, Kuo, and Eisenberg, 1995).

Together, it is highly likely that the “black” hippocampus and “tan” amygdala networks are related to brain development and function.

Table 3.2: GO enrichment results for the “black” network from Hippocampus. Among the enriched GO terms, it was most interesting to identify a number of brain developmental related GO terms such as “central nervous system development”, “axon ensheathment in central nervous system”, “glutamate metabolic process” and “positive regulation of gliogenesis”. Surprisingly, GO related to immune systems were also observed “positive regulation of production of molecular mediator of immune response”.

term_ID	description	p-value
GO:0019752	carboxylic acid metabolic process	4.92×10^{-6}
GO:0007417	central nervous system development	5.94×10^{-5}
GO:0002821	positive regulation of adaptive immune response	6.12×10^{-5}
GO:0006082	organic acid metabolic process	1.03×10^{-3}
GO:0032291	axon ensheathment in central nervous system	1.86×10^{-3}
GO:1901565	organonitrogen compound catabolic process	1.99×10^{-3}
GO:0006536	glutamate metabolic process	3.54×10^{-3}
GO:0021762	substantia nigra development	3.73×10^{-3}
GO:0044281	small molecule metabolic process	4.34×10^{-3}
GO:0030194	positive regulation of blood coagulation	4.59×10^{-3}
GO:0009607	response to biotic stimulus	6.14×10^{-3}
GO:0002702	positive regulation of production of molecular mediator of immune response	6.21×10^{-3}
GO:0034103	regulation of tissue remodeling	6.21×10^{-3}
GO:0014015	positive regulation of gliogenesis	7.47×10^{-3}
GO:0098542	defense response to other organism	7.95×10^{-3}
GO:0019835	cytolysis	8.72×10^{-3}

3.4. DISCUSSION

Table 3.3: GO enrichment results for the “tan” network from Amygdala. Unlike the “black” network, only a small number of GO terms were enriched. However, these GO terms are relatively specific to amine/ammonium ion metabolism. Interestingly, ammonium ion are essential to the synthesis of glutamine from glutamate, suggesting that this network might be relate to the glutamate system.

term.ID	description	p-value
GO:0097164	ammonium ion metabolic process	1.37×10^{-3}
GO:0044106	cellular amine metabolic process	4.2×10^{-3}
GO:0009308	amine metabolic process	5.41×10^{-3}
GO:0046519	sphingoid metabolic process	6.01×10^{-3}

Associate Co-expression network with PGC schizophrenia data

Although the co-expression network were extremely interesting for their expression pattern and functional enrichment in brain development and function related GO terms, there were no evidence of their involvement nor importance in schizophrenia. Therefore it is of particular interest for us to test whether if genes within these co-expression networks were associated with schizophrenia.

First, gene base p-value of 18,622 genes were calculated using p-values from the PGC schizophrenia working group(Stephan Ripke et al., 2014). Gene set enrichment analysis were then performed using MAGMA(Leeuw et al., 2015) to test whether if there genes within the “black” hippocampus and “tan” amygdala networks were significantly associated with schizophrenia.

Based on the self-contained gene set enrichment analysis, genes within both networks were significantly associated with schizophrenia with p-value of 1.38×10^{-41} for the “tan” amygdala network and 2.70×10^{-74} for the “black” hippocampus network. These suggest that these networks might be disrupted in schizophrenia patients.

Partitioning of Heritability

3.4 Discussion

Chapter 4

Heritability of Response to antipsychotic treatment

Important to schizophrenia research

4.1 Introduction

Here we try to use Beatrice's data and estimate the heritability explained in drug response.
Should also repeat the region-wise heritability

4.2 Methodology

4.3 Result

4.4 Discussion

Chapter 5

Conclusion

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Supplementary Materials

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Appendix