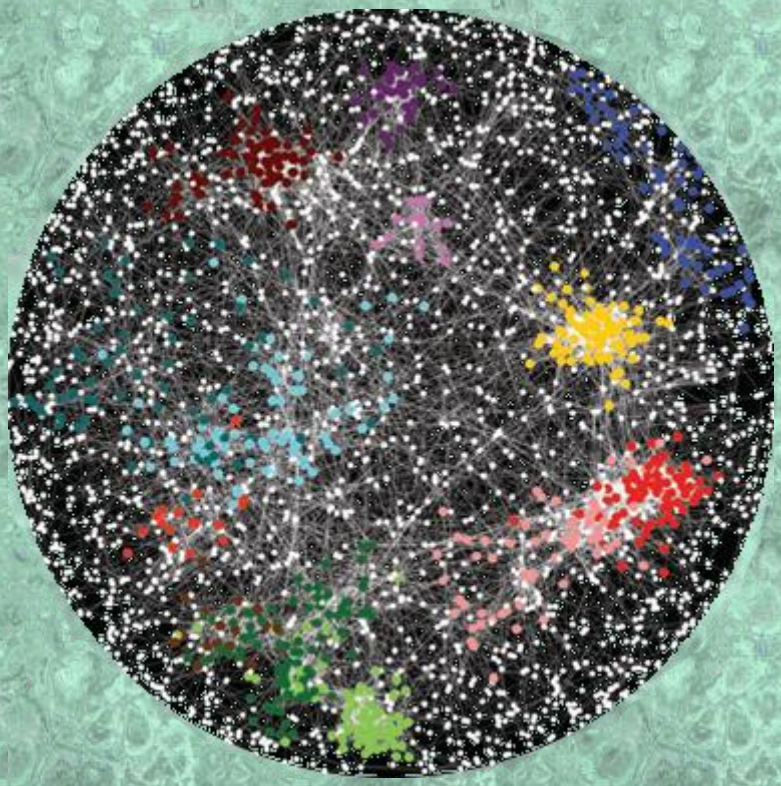
Genomic Association Screening Methodology for High-Dimensional and Complex Data Structures

Detecting n-Order Interactions

Jestinah M. Mahachie John



Promotor: Prof. Dr. Dr. Kristel Van Steen

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To the love of my life, Genesis Chevure

To the first fruit of my womb and currently the only one, Takunda Chevure

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Above all, I give glory, adoration and honour to the Almighty God!

Summary

We developed a data-mining method, Model-Based Multifactor Dimensionality Reduction (MB-MDR) to detect epistatic interactions for different types of traits. MB-MDR enables the fast identification of gene-gene interactions among 1000nds of SNPs, without the need to make restrictive assumptions about the genetic modes of inheritance. This thesis primarily focused on applying Model-Based Multifactor Dimensionality Reduction for quantitative traits, its performance and application to a variety of data problems. We carried out several simulation studies to evaluate quantitative MB-MDR in terms of power and type I error, when data are noisy, non-normal or skewed and when important main effects are present.

Firstly, we assessed the performance of MB-MDR in the presence of noisy data. The error sources considered were missing genotypes, genotyping error, phenotypic mixtures and genetic heterogeneity. Results from this study showed that MB-MDR is least affected by presence of small percentages of missing data and genotyping errors but much affected in the presence of phenotypic mixtures and genetic heterogeneity. This is in line with a similar study performed for binary traits. Although both Multifactor Dimensionality Reduction (MDR) and MB-MDR are data reduction techniques with a common basis, their ways of deriving significant interactions are substantially different. Nevertheless, effects on power of introducing error sources were quite similar. Irrespective of the trait under consideration, epistasis screening methodologies such as MB-MDR and MDR mainly suffer from the presence of phenotypic mixtures and genetic heterogeneity.

Secondly, we extensively addressed the issue of adjusting for lower-order genetic effects during epistasis screening, using different adjustment strategies for SNPs in the functional SNP-SNP interaction pair, and/or for additional important SNPs. Since, in this thesis, we restrict attention to 2-locus interactions only, adjustment for lower-order effects always (and only) implies adjustment for main genetic effects. Unfortunately most data dimensionality reduction techniques based on MDR do not explicitly require that lower-order effects are included in the 'model' when investigating higher-order effects (a prerequisite for most traditional, especially regression-based, methods). However, epistasis results may be hampered by the presence of significant lower-order effects. Results from this study showed hugely increased type I errors when main effects were not taken into account or were not properly accounted for. We observed that additive coding (the most commonly used coding in practice) in main effects adjustment does not remove all of the potential main effects that deviate from additive genetic variance. In addition, also adjusting for main effects prior to

MB-MDR (via a regression framework), whatever coding is adopted, does not control type I error in all scenarios. From this study, we concluded that correction for lower-order effects should preferentially be done via codominant coding, to reduce the chance of false positive epistasis findings. The recommended way of performing an MB-MDR epistasis screening is to always adjust the analysis for lower-order effects of the SNPs under investigation, "on-the-fly". This correction avoids overcorrection for other SNPs, which are not part of the interacting SNP pair under study.

Thirdly, we assessed the cumulative effect of trait deviations from normality and homoscedasticity on the overall performance of quantitative MB-MDR to detect 2-locus epistasis signals in the absence of main effects. Although MB-MDR itself is a non-parametric method, in the sense that no assumptions are made regarding genetic modes of inheritance, the data reduction part in MB-MDR relies on association tests. In particular, for quantitative traits, the default MB-MDR way is to use the Student's *t*-test (steps 1 and 2 of MB-MDR). Also when correcting for lower-order effects during quantitative MB-MDR analysis, we intrinsically maneuver within a regression framework. Since the Student's *t*-statistic is the square root of the ANOVA F-statistic. Hence, along these lines, for MB-MDR to give valid results, ANOVA assumptions have to be met. Therefore, we simulated data from normal and non-normal distributions, with constant and non-constant variances, and performed association tests via the student's *t*-test as well as the unequal variance *t*-test, commonly known as the Welch's *t*-test. At first somewhat surprising, the results of this study showed that MB-MDR maintains adequate type I errors, irrespective of data distribution or association test used. On the other hand, MB-MDR give rise to lower power results for non-normal data compared to normal data. With respect to the association tests used within MB-MDR, in most cases, Welch's *t*-test led to lower power compared to student's *t*-test. To maintain the balance between power and type I error, we concluded that when performing MB-MDR analysis with quantitative traits, one ideally first rank-transforms traits to normality and then applies MB-MDR modeling with Student's *t*-test as choice of association test. Clearly, before embarking on using a method in practice, there is a need to extensively check the applicability of the method to the data at hand. This is a common practice in biostatistics, but often a forgotten standard operating procedure in genetic epidemiology, in particular in GWAI studies.

In addition to the presentation of extensive simulation studies, we also presented some MB-MDR applications to real-life data problems. These analyses involved MB-MDR analyses on

quantitative as well as binary complex disease traits, primarily in the context of asthma/allergy and Crohn's disease. In two of the presented analyses, MB-MDR confirmed logistic regression and transmission disequilibrium test (TDT) results. Part of the aforementioned methodological developments was initiated on the basis of observations of MB-MDR behavior on real-life data.

Both the practical and theoretical components of this thesis confirm our belief in the potential of MB-MDR as a promising and versatile tool for the identification of epistatic effects, irrespective of the design (family-based or unrelated individuals) and irrespective of the targeted disease trait (binary, continuous, censored, categorical, multivariate). A thorough characterization of the different faces of MB-MDR this versatility gives rise to is work in progress.

Résumé

Nous avons développé une méthode d'exploration de données, à savoir la Réduction de dimensionnalité multifactorielle basée sur un modèle Model-Based Multifactor Dimensionality Reduction (MB-MDR) afin d'identifier des interactions épistatiques pour différents types de caractères. La méthode MB-MDR permet une identification rapide des interactions entre gènes parmi 1000 marqueurs ou SNP, sans devoir faire des hypothèses restrictives concernant les modes de transmission génétique. Cette thèse porte principalement sur l'application de la réduction de dimensionnalité multifactorielle basée sur un modèle MB-MDR aux caractères quantitatifs, leur performance et leur application à une série de problèmes relatifs aux données. Nous avons mené une série d'études de simulation en vue d'évaluer la MB-MDR quantitative en termes de performance et d'erreur de type I, dans le cas où les données sont bruyantes, anormales ou faussées et en présence d'effets importants principaux.

Premièrement, nous avons évalué la performance de la MB-MDR en présence de données bruyantes. Les sources d'erreur considérées étaient les génotypes manquants, les erreurs de génotype, les mélanges phénotypiques et l'hétérogénéité génétique. Les résultats de cette étude ont démontré que la MB-MDR est influencée dans une moindre mesure en présence d'un faible pourcentage de données manquantes et d'erreurs de génotype. Elle est toutefois fortement influencée en présence de mélanges phénotypiques et d'une hétérogénéité génétique. Ces résultats sont conformes à une étude similaire menée dans le cadre des caractères binaires. Même si la Réduction de dimensionnalité multifactorielle (MDR) et la MB-MDR sont des techniques de réduction de données sur base commune, leur mode de dérivation d'interactions significatives est substantiellement différent. Toutefois, les conséquences constatées sur la capacité d'introduction d'erreurs sources sont assez similaires. Indépendamment du caractère en question, les méthodes de détection de l'épistasie comme la MB-MDR et MDR sont principalement gênées par la présence de mélanges phénotypiques et de l'hétérogénéité génétique.

Deuxièmement, nous avons largement traité du problème que constitue l'adaptation des effets génétiques d'ordre inférieur pendant la détection de l'épistasie, moyennant l'utilisation de différentes stratégies d'adaptation pour les marqueurs dans la paire interactive SNP-SNP fonctionnelle et/ou les principaux marqueurs supplémentaires. Comme nous nous sommes limités dans cette thèse aux interactions à 2 loci, l'adaptation des effets d'ordre inférieur implique toujours (et uniquement) une adaptation des principaux effets génétiques.

Malheureusement, la plupart des techniques de réduction de dimensionnalité basées sur la MDR ne nécessitent pas explicitement la présence d'effets d'ordre inférieur dans le 'modèle' lors de l'étude des effets d'ordre supérieur (une condition indispensable pour la plupart des méthodes traditionnelle à base de régression). Cependant, les résultats épistasiques peuvent être entravés par la présence d'effets significatifs d'ordre inférieur. Les résultats de cette étude ont montré une importante hausse des erreurs de type I lorsque les principaux effets ne sont pas pris en compte ou correctement comptabilisés. Nous avons également constaté que le codage additif (le code le plus utilisé en pratique) dans l'adaptation des effets principaux n'annule pas tous les effets principaux potentiels découlant de la variance génétique additive. Par ailleurs, l'adaptation des principaux effets précédant la MB-MDR (sur la base d'un modèle à régression), peu importe le codage utilisé, ne contrôle pas l'erreur de type I dans tous les cas. Dans le cadre de cette étude, nous avons conclu que la correction des effets d'ordre inférieur devrait se faire de préférence moyennant le codage codominant, afin de réduire le risque de résultats épistasiques faussement positifs. Il est conseillé de réaliser une détection épistasique MB-MDR en adaptant toujours l'analyse en fonction des effets d'ordre inférieur des marqueurs étudiés, "on-the-fly". Cette correction évite la surcorrection des autres marqueurs, qui ne font pas partie de la paire SNP interactive étudiée.

Troisièmement, nous avons étudié l'effet cumulé des déviations de caractère par rapport à la normalité et à l'homoscédasticité sur l'ensemble des performances de la MB-MDR afin d'identifier l'épistasie entre 2 loci en l'absence des principaux effets. Même si la méthode MB-MDR est une technique non paramétrique, autrement dit aucune hypothèse n'est faite concernant les modes de transmission génétique, la réduction des données dans la MB-MDR dépend de tests d'association. Plus particulièrement en ce qui concerne les caractères quantitatifs, la méthode MB-MDR standard à utiliser est le test t de Student (étapes 1 et 2 de la MB-MDR). Lorsque nous adaptons les effets d'ordre inférieur pendant l'analyse MB-MDR quantitative, nous agissons de manière intrinsèque dans un cadre de régression. Le test t de Student étant la racine carrée du test f ANOVA, les hypothèses ANOVA doivent dès lors être rencontrées pour que la MB-MDR donne des résultats valides. C'est pourquoi, nous avons simulé des données de distributions normales et anormales, avec des variances constantes et non constantes et avons réalisé des tests d'association via le test t et la variance inégale du test t, mieux connu sous le nom de test de Welch. Il est étonnant de voir à première vue, que les résultats de cette étude indiquent que la méthode MB-MDR permet des erreurs de type I

appropriées, indépendamment de la distribution des données ou du test d'association utilisé. D'un autre côté, la méthode MB-MDR donne lieu à des résultats moins performants pour les données anormales en comparaison avec les données normales. Conformément aux tests d'association utilisés dans le cadre de la méthode MB-MDR, le test de Welch est le plus souvent, moins performant que le test t. Afin de garder l'équilibre entre les performances et l'erreur de type I, nous avons conclu que lorsque nous réalisons une analyse MB-MDR avec des caractères quantitatifs, il convient idéalement de ramener les caractères à la normalité avant d'appliquer une MB-MDR avec comme test d'association, le test t de Student. Il est évident qu'il convient de vérifier de manière approfondie, avant d'utiliser concrètement une méthode, son applicabilité aux données en question. Il s'agit là d'une pratique commune des biostatistiques et d'une procédure d'exploitation standard souvent oubliée dans l'épidémiologie génétique, en particulier dans les études GWAI.

En outre de la présentation des vastes études de simulation, nous avons également appliqué la méthode MB-MDR à certains problèmes de données réels. Ces analyses comprennent des analyses MB-MDR faites sur des traits de maladie complexe quantitative et binaire, premièrement dans le cas de l'asthme/allergie et ensuite dans le cas de la maladie de Crohn. Dans deux des analyses présentées, la méthode MB-MDR a confirmé les résultats du test de régression logistique et du TDT. Le chapitre sur les développements méthodologiques susmentionnés a été initié sur la base d'observations de la méthode MB-MDR sur des données réelles.

Les composantes pratiques et théoriques de cette thèse confirment notre foi dans le potentiel du MB-MDR comme un moyen prometteur et polyvalent dans le cadre de l'identification des effets épistatiques, indépendamment du concept (familial ou anonyme) et des traits de la maladie étudiée (binaires, continus, censurés, catégoriques, multi-varié). Cette polyvalence qui créé les différentes facettes de la MB-MDR est un travail qui n'est pas encore achevé.

List of Acronyms

AIC	Akaike's information criterion
ANOVA	Analysis of variance
CD	Crohn's disease
CNV	Copy number variation
DNA	Deoxyribonucleic acid
FWER	Family-wise error rate
GE	Genotyping error
GH	Genetic heterogeneity
GWA	Genome-wide association
GWAI	Genome-wide association interaction
HWE	Hardy-Weinberg equilibrium
IF	Impact factor
LD	Linkage disequilibrium
MAF	Minor allele frequency
MB-MDR	Model-based multifactor dimensionality reduction
MDR	Multifactor dimensionality reduction
MG	Missing genotype
MR	Multiple regression
PM	Phenotypic mixture
RNA	Ribonucleic acid
SNP	Single-nucleotide polymorphism
SR	Single regression
ST	Student’s t-test
UC	Ulcerative colitis
WT	Welch’s t-test

Table of Contents

List of Figures

PART 1

PART 2

PART 3

List of Tables

PART 1

PART 2

PART 1

INTRODUCTION AND AIMS

Chapter 1: Introduction

1.1 Complex Diseases

Healthy functioning of the immune system is of paramount importance to everyone since it controls the ability to fend off illness and disease. What really causes disease and why it is that some people develop cancers while others develop heart attacks or why some people rather develop debilitating disorders such as arthritic conditions, Crohn's disease, Alzheimer disease, asthma, diabetes, multiple sclerosis, Parkinson disease, osteoporosis, glaucoma, depression, during their life-time, are easily formulated questions, yet with non-trivial answers. In contrast to diseases that are driven by a single gene (i.e., monogenic disease), the aforementioned diseases have a multifactoral genetic underpinning (i.e., polygenic). By definition, the etiology of *complex diseases* usually involves a combination of genetic, environmental, and lifestyle factors, most of which have not yet been identified [1]. This combination of different gene varieties, possibly having a modifying effect on each other (as the result of gene-gene interactions) or the potential modifying effect of the surrounding environment (as the result of gene-environment interactions) may lead to the development or progression of disease in some individuals but not in others. Complex diseases are indeed "complex" and it leaves no doubt that finding the causal mechanisms of a complex disease will be a challenge in genetic epidemiology for several years to come [2]. In an attempt to characterize genetic contributors to (complex) diseases, there exist several tools to collect genetic data, analyze or process in order to derive useful information from them.

This chapter presents a brief overview of important developments in genetic association studies that are relevant for the sequel of this thesis. We start by outlying how genetic information can be captured and summarize commonly used criteria to check data quality. In Section 1.8, we motivate studying gene-gene interactions and describe the foundations on which this work is built.

1.2 Human Genetic Information

Individual genomes stored in the sequence of *deoxyribonucleic acid* (DNA) can differ greatly, from single-letter changes to complex structural differences over chunks of up to a million base pairs of genetic code [3, 4]. This genetic code is made up of four chemical

nucleotide bases: adenine (A), guanine (G), cytosine (C), and thymine (T). Several sources of genomic variation in humans exist causing uniqueness from one individual to the other.

1.2.1 Single Nucleotide Polymorphisms

Single nucleotide polymorphism (SNP) refers to a single base pair change that is variable across the general population at a frequency of at least 1%. Each SNP represents a difference in a single DNA building block. There are two types of nucleotide base substitutions resulting in SNPs: 1) a *transition* substitution which occurs between purines (A, G) or between pyrimidines (C, T) and 2) a *transversion* substitution which occurs between a purine and a pyrimidine. The former constitutes two thirds of all SNPs [5]. A SNP in a coding region may be referred to as *synonymous* if the substitution causes no amino acid change to the protein it produces or as *non-synonymous* if the substitution results in an alteration of the encoded amino acid. The different bases, either A, T, C or G, present at a SNP location are known as *alleles*. In humans, most SNPs are *bi-allelic*, indicating there are two possible bases at the corresponding site within a gene (e.g. *A* and *a*). From these SNP bases, three allele combinations known as *genotypes* in a population can be observed: the homozygous wildtype, *AA*, heterogeneous, *Aa* and homozygous rare, *aa*. In this thesis, we will use SNPs as genetic markers.

1.2.2 Copy Number Variations

While most initial studies of genetic variation concentrated on individual nucleotide sequences (SNPs), investigators have also found that large-scale changes involving loss or gain of the DNA sequence occur in many locations throughout the genome. Structural variations such as insertions, deletions, inversions, duplications, translocations and copy number variations (CNVs) result in changes in the physical arrangement of genes on chromosomes. Redon et al. [6] defined a CNV as a DNA segment of one kilobase or larger that is present at a variable copy number in comparison with a reference genome. During the past several years, hundreds of new variations in repetitive regions of DNA have been identified, leading researchers to believe that CNVs are also an important component of genomic diversity [7, 8].

1.2.3 Epigenetics

Epigenetics is another type of genetic variation used to describe heritable features that control the functioning of genes within an individual cell but do not constitute a physical change in the corresponding DNA sequency [9]. This type of variation arises from chemical tags that attach to DNA and affect how it gets read. At some alleles, the epigenetic state of the DNA and associated phenotype can be inherited transgenerationally [10, 11].

1.3 Quality Control

It is important that prior to any statistical analysis, a quality control step is carried out. This step is needed to carefully consider and account for potential marker errors that could lead to false significant association [12]. Essential criteria used for genomic data quality control involve *Hardy-Weinberg equilibrium* handling*, minor allele frequency* checks and *genotype call rate* control. For more details about the standard quality control filters for genome-wide association studies, we refer to the *Travemunde Criteria* [13, 14]. Our lab is currently developing a minimal protocol for genome-wide association interaction screening.

1.3.1 Hardy-Weinberg Equilibrium

Hardy-Weinberg equilibrium (HWE) refers to the independence of alleles at a single site between two homologous chromosomes. Unless specific disturbing influences (e.g. non-random mating, random genetic drift) are introduced the allele and genotype frequencies remain constant from generation to generation [15]. HWE is recommended to be checked only in founders or in controls due to the fact that departures from HWE may arise in diseased individuals if a genuine association exists between the SNP and the disease [16-20]. SNPs that are out of HWE (after multiple correction) are excluded from further analysis. For a bi-allelic locus, the *de Finetti diagram* is extensively used to graphically represent relationships between genotype frequencies [19, 21]. No consensus exists on the appropriate threshold for HWE *p*-values [22].

1.3.2 Minor Allele Frequency

The minor allele frequency (MAF) refers to the frequency of the least common allele at a variable site. Most genetic association studies are based on the *common disease-common variant* (CDCV) hypothesis. With large-scale sequencing, a shift to the *common disease-rare*

variant (CDRV) hypothesis is gradually taking place [23]. In the context of former CDCV hypothesis, rare SNPs with MAFs less than 5% are often excluded from analysis.

1.3.3 Genotype Call Rate

The (per SNP) genotype call rate refers to proportion of genotypes per marker with non-missing data (i.e. the proportion of observed genotype counts). It is common practice to remove markers with a call rate less than 95% [12, 24-26].

1.4 The Principles of Genetic Association

A genetic association refers to statistical relationships in a population between an individual's genetic information and a phenotype. The genetic association can be either direct or indirect, depending on whether the allele under investigation directly influences the phenotype or whether the allele is in *linkage disequilibrium* (LD) with the disease-predisposing mutation [27]. LD refers to the non-random association between two alleles at two loci on a chromosome in a natural breeding population [28]. An important advance towards enabling efficient genetic association studies was the determination of LD patterns on a genome-wide scale through the HapMap project [29].

According to Foulkes [9], genetic association studies can be roughly divided into four categories: *candidate polymorphism, candidate gene, fine mapping and whole genome-wide scans*. Two fundamentally different designs are used in genetic association studies: family-based designs and population designs that use unrelated individuals (see Table 2 of [30] for a comprehensive list of commonly used designs).

1.4.1 Candidate Polymorphism Studies

Candidate polymorphism studies involve investigations of associations between a marker and a trait for which there is an a priori hypothesis about functioning. These studies rely on prior scientific evidence suggesting that the set of SNPs under investigation is relevant to the disease trait. The goal of these studies is to determine whether a given SNP or a set of SNPs is functional and has a direct influence on the trait.

1.4.2 Candidate Gene Studies

Unlike candidate polymorphism studies which look at SNPs irrespective of common location on a gene or not, candidate gene studies involve multiple SNPs within a single gene. The

choice of SNPs depends on predefined linkage disequilibrium blocks. The SNPs being studied in these studies are not necessarily functional. Recall that the underlying criterion linked to LD is that the SNPs under investigation capture information about the genetic variability of the gene under consideration, though the SNPs may not serve as the true disease-causing variants. Currently, candidate gene studies are mainly used to validate findings from genome-wide association studies as well as further exploring of additional associations based on clinical variables (see Section 1.4.4).

1.4.3 Fine Mapping Studies

Fine-mapping involves the identification of markers that are very tightly linked to a targeted gene. These studies aim to determine precisely where on the genome the mutation that causes the disease is positioned. Within the context of mapping studies, the term *quantitative trait loci* (QTL) is used to refer to stretches of DNA containing or linked to the genes that underlie a quantitative trait.

1.4.4 Genome-wide Association Studies

Like candidate gene approaches, genome-wide association (GWA) studies aim at identifying associations between genetic markers and a trait. However, GWA studies tend to be less hypothesis driven and involve characterization of a much larger number of SNPs [9]. The shift from candidate gene to GWA studies has been made possible through the completion of the Human Genome Project in 2003 [31] and the International HapMap Project in 2005 [32]. GWA studies have been successful in identifying genetic associations with more than 1600 published GWA studies on SNPs at a genome-wide significance level $p \leq 5 \times 10^{-8}$ for more than 280 traits [33, 34].

1.5 Sequencing

Recent advances in sequencing technology has enabled the identification of rare variants (MAF<5%) [35]. Apart from SNPs, rare variants have been reported to contribute to the genetic variation of complex diseases as well [36]. *Sequencing* is the process of reading the nucleotide bases in a DNA molecule hereby looking at all portions of the genome, not just those that include instructions for making proteins [37]. Sequencing a person's entire genetic code is known as *whole-genome sequencing*. *Exome sequencing* refers to the processing of sequencing only the coding regions of the genome. The exome makes up about 1% of the

genome [38]. Although exome sequencing has recently been shown to expedite disease gene discovery, it misses non-coding variation and some structural variations [39-44]. As the cost difference between exome and whole-genome sequencing shrinks, additional methods are needed to analyze the wealth of information whole-genome sequencing provides [45, 46].

1.6 Genetic Models in GWA Studies

In order to determine the appropriate test for association, a genetic model must first be specified [47]. One of the important components of a genetic model involves the *inheritance pattern*, the transmission of material from parent to offspring. When the transmission involves genetic material, the inheritance is termed *genetic inheritance*. There are several modes of inheritance (genetic and non-genetic) and these can be categorized into three groups: *single gene or Mendelian* (genetic conditions caused by a mutation in a single gene follow predictable patterns of inheritance within families), *Multifactorial* (inheritance pattern resulting from an interplay between genetic factors and environmental factors as in complex diseases) and *Mitochondrial* (inheritance from the mother's egg) [48]. Mendelian inheritance can be further categorized into *autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive*. Dominant conditions are expressed in individuals who have just one copy of the mutant allele whereas recessive conditions are clinically manifest only when an individual has two copies of the mutant allele. For X-linked inheritance, the gene causing the trait or the disorder is located on the X chromosome [49].

However, due to the absence of sufficient biological understanding of genetically complex diseases, the true underlying mode of inheritance is rarely known [50, 51]. Researchers customarily test several genetic models and choose the most parsimonious model to explain the data at hand [52, 53]. In other words, the choice is often driven by convention or convenience. Most used genetic models include, but are not limited to additive, recessive, dominant, codominant, multiplicative [54]. These models are defined based on another component of genetic models called the *penetrance parameter* of the trait allele. Penetrance parameters specify the relationship between genotype and trait [55]. For a dichotomous trait, a penetrance parameter is defined for each genotype as the $P(trait|genotype)$. For a quantitative trait, Y, the penetrance function describes the distribution of the trait conditional on an individual's genotype, $f(Y|genotype)$.

In population association studies (e.g. case-control studies), the risk of disease is interpreted differently depending on the genetic model used. Under the *codominant model*, the risk of

disease when having two copies of affected allele is arbitrarily different from having a single copy. Under the *dominant model*, a single affected allele increases disease risk whereas under the *recessive model*, two copies of the affected allele are required for increased risk. Under *the additive model*, having two affected alleles have twice increased risk as compared to having a single affected allele. Under the *multiplicative model*, the increased risk of having two affected alleles is a square of having a single affected allele [56, 57]. The analysis for the multiplicative model is performed by allele not genotype and requires both case and control genotypes to be in be in Hardy–Weinberg Equilibrium [58].

In the context of quantitative traits, phenotype variability can be attributed to genetic variation and environmental variation. The proportion of phenotypic variance that is attributable to genotypic variance is known as *heritability* [59]. In quantitative GWA studies, genetic variance is often further divided into additive and dominance variance. Variance in phenotype can also result from epistatic effects and hence the variance decomposition can be extended to multiple loci. For instance, for a two-locus interaction, the epistatic variance decomposition can be attributed to *additive-additive* (interactive effect of two alleles, one from each locus), *additive-dominance* (interaction effects of three alleles, one from one locus and two from the other) and *dominance-dominance* (interactive combinations of four alleles, two from each locus) [60].

1.7 From GWA Studies to Genome-wide Association Interaction Studies

Hundreds of millions of dollars have been spent on GWA studies and more than 400 susceptibility regions identified but still most of the genetic variance in risk or quantitative trait for most common diseases remains undiscovered. Genome-wide association studies have led to the identification of >1 600 loci harboring genetic variants associated with >280 common human diseases and traits [34].

Although great progress in genome-wide association studies has been made, the significant SNP associations identified by GWA studies account for only a few percent of the genetic variance, leading many to question where and how we can find the missing heritability. The proportion of heritability apparently explained by GWA studies has grown (to 20–30% in some well-studied cases and >50% in a few), but, for most traits, the majority of the heritability remains unexplained [61]. This has led the field of genetics to be faced with a dilemma now known as the *missing heritability problem* [62]. Several possible explanations of this “missing heritability” have been proposed [23, 35, 36, 63-65]. These include *alleles*

with small effects (small effects virtually eliminates any detectable signal and requires unfeasibly large sample sizes to allow detection), *rare variants* (when GWA studies began, the field was dominated by the simple common disease–common variant hypothesis), *population differences* (most genetic variations are associated with the geographical and historical populations in which the mutations first arose), *disease heterogeneity* (some diseases are actually simply collections of symptoms, which may stem from multiple, distinct genetic causes), copy number variation (see Section 1.2.2), epigenetic inheritance (see Section 1.2.3), and lastly the focus of this thesis, *epistatic interactions* (genes associated with the same disease, compared to genes associated with different diseases, more often tend to share a protein-protein interaction and a gene ontology biological process).

1.8 Genome-wide Association Interaction (GWAI) Studies

The complexity of genetics of human complex diseases can be largely attributed to epistatic or gene-gene interactions [66]. The presence of gene-gene interactions is of particular concern in complex disease genetics because if the effect of one locus is altered or masked by effects at another locus, power to detect the first locus is likely to be reduced and elucidation of the combination of independent effects at the two loci will be hindered by their interaction. If more than two loci are involved, the situation is likely to be further complicated by the possibility of complex multi-way interactions among some or all of the contributing loci.

The term ‘epistasis’ was initially used by Bateson [67] to refer to a masking effect whereby a variant or allele at one locus prevents the variant at another locus from manifesting its effect. In other words, it refers to a distortion of Mendelian segregation ratios due to one gene making the effect of another. Few years later, Fisher [68] defined epistasis in a statistical way in terms of deviations from a model of multiple additive effects with respect to a quantitative phenotype.

Note that, depending on the scale used to assess these deviations, different definitions for epistasis are implied. Moreover, the same terminology has been used in different areas with a totally different meaning. What is meant by epistasis may vary depending on whether biologists, epidemiologists, statisticians, or human and quantitative geneticists are involved [69]. This has led in the past to a lot of confusion and uncertainty about how to best approach the problem of epistasis identification.

1.8.1 Biological Epistasis and Statistical Epistasis

Epistasis can be viewed from two major perspectives, biological and statistical, each derived from and leading to different assumptions and research strategies. The two perspectives have been reviewed in detail by Moore and Williams [70]. We use this article as an anchor article for this and the next section to illustrate biological and statistical epistasis.

It should be noted that biological epistasis results from physical interactions among biomolecules (e.g. DNA, RNA, proteins, enzymes, etc.) within gene regulatory networks and biochemical pathways and occurs at the cellular level in an individual. On the other hand, statistical epistasis (in the Fisher sense, extended to non-qualitative traits) occurs at the population level and is realized when there is inter-individual variation in DNA sequences (vertical bars, Figure 1.1). Here it is assumed that the relationship between multi-locus genotypes and phenotypic variation in a population is not predictable based solely on the actions of the genes considered singly. The existence of biological epistasis may go undetected at a population level, due to a variety of reasons, including power of the statistical analysis approach. Hence, under "optimal" conditions, biological variation may be viewed as sufficient for the statistical detection of epistasis. Biological epistasis can nevertheless occur in the absence of statistical epistasis when every individual sampled from a population is the same with respect to their DNA sequence variations and biomolecules (circle, square and triangle, Figure 1.1). Vice versa, evidence for statistical epistasis may not always be easily translated into biological epistasis. The key challenge is to develop methodologies that can bridge or narrow the gap between these two viewpoints. How to do this, is still less clear [20].

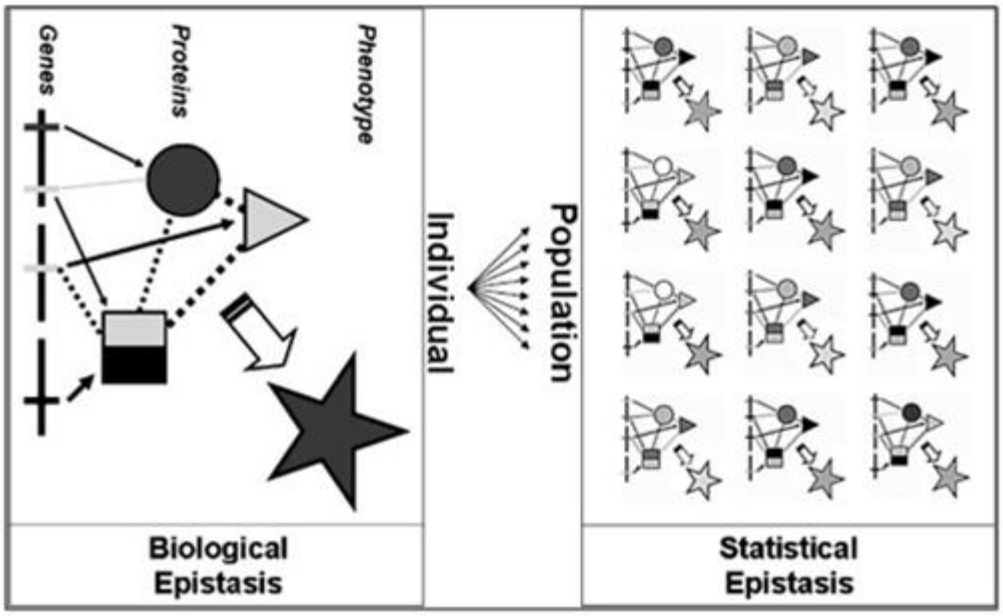

Figure 1. 1 The conceptual relationship between biological and statistical epistasis. *Source: Moore and Williams* [70].

1.8.2 Importance of Epistasis

The limitations of data collection when considering humans (ideally several 1000nds of homogeneous samples are obtained to unravel disease-related gene-gene interactions [71-73]), as well as the inability to perform rigorous genetic experiments on human subjects, hampers the quest for knowledge about genetic mechanisms operating on human complex disease traits. The progress in understanding human disease genes owes much to research in experimental organisms [74-76]. Animal models have greatly improved our understanding of the cause and progression of human genetic diseases and have proven to be a useful tool for discovering targets for therapeutic drugs [77].

An important lesson learnt from model organisms is that orthologous genes are ubiquitously present in living organisms (i.e. there are certain genes that all living organisms from a common ancestor have because they perform very basic life functions). This knowledge has been extremely important in human genetics. The function of many identified human disease genes has been inferred from functional information about its ortholog in model organisms [78, 79]. Of human genes, 50% are orthologous with yeast. In addition, the near-complete sequence of the mouse genome reveals that 99% of mouse genes turn out to have analogues in humans and that most mouse and human ortholog pairs have a high degree of protein sequence identity with a mean amino acid identity of 78.5% [80]. Since living organisms

clearly share some similar biochemical processes, evidence in one organism may give important clues about functioning and existing processes in another.

Just as model organisms have been instrumental in defining the roles of genes and the structure of genetic pathways that are important for human disease in GWA studies, they are equally useful in defining the principles of epistasis or to detect gene-gene interactions. Moreover, experimental organisms may be even more useful in the context of gene-gene interactions than for the characterization of the functions of individual genes, because – to date - the power resulting from genetic tractability (i.e. one can take genes out of an organism or put genes into it very easily) is often compounded in studies of gene interaction [76]. Mice is the most genetically tractable of mammalian species [81].

Studies in model organisms have shown that epistatic interactions may occur frequently and can even involve more than two loci [82, 83]. For instance, novel loci that act through epistatic pathways have been identified through multidimensional scans of complex traits in mice [84, 85], Drosophila [86], chicken [83, 87], plants [88, 89] and rats [90, 91]. These studies suggest that multiple interacting genes can influence complex phenotypes often in the absence of significant single-locus effects [92]. However, other similar studies have reported only low levels of epistasis or no epistasis at all, despite being thorough and involving large sample sizes [91, 93, 94]. This clearly indicates the complexity with which multifactorial traits are regulated; no single mode of inheritance can be expected to be the rule in all populations and traits.

A handful of evidences exist for humans. Some of the existing ones have been cited in Phillips [95]. Examples include diabetes [96], coronary artery disease [97], bipolar effective disorder [98], and autism [99]. To date, only for some of the reported findings additional support could be provided by functional analysis, as was the case for multiple sclerosis. Here, Gregersen et al. [100] found evidence that natural selection might be maintaining linkage disequilibrium between several different histocompatibility loci (*DR2a* and *DR2b*) known to be associated with multiple sclerosis. A more recent example involves Alzheimer's disease (AD). Combarros et al. [101] replicated an interaction between IL-6 and IL-10 on AD that was found in their preliminary study in the Rotterdam dataset and had been reported earlier by Infante et al. [102].

Finally, looking into epistatic interactions might lead to the uncovering of cryptic genetic variation, hereby enhancing genetic heritability explanation [103-105]. This is turn paves the

way to a better understanding of the mechanism of disease-causing genetic variants and of the role epistasis may play in explaining human variation and human health. In this thesis, we make the assumption that epistasis is a natural thing to occur but we remark that there are also complicating factors that make its detection difficult (discussed in the next section).

1.8.3 Statistical methods to detect epistasis

The number of identified epistatic effects in humans (not necessarily replicated!), showing susceptibility to common complex human diseases, follows a steady growth curve [106, 107], due to the growing number of toolbox methods and approaches.

Most of the developed methods to detect epistasis have not yet been successfully applied in the context of genome-wide real-life data (success here defined as biologically or clinically relevant), mainly due to two issues; 1) GWAI methods are computationally intensive and 2) a large number of samples are needed to be translated into sufficient power to detect epistatic effects from 100 thousands of SNPs, which are usually not available. In the absence of efficient computational algorithms or a powerful IT environment, GWAI studies may become practically infeasible, since the number of possible SNP-SNP interactions grows exponentially with the number of involved SNPS. One way to deal with the exponential increase is to pre-select "interesting" regions of the genome, hereby reducing the computational burden. Typical for large-scale epistasis studies, such as GWAI studies, is that one has to deal with a 'small *n* big *p*' problem, where the number of samples (*n*) is much smaller than the number of variables (*p*) [20, 108], potentially giving rise to *curse of dimensionality* problems. The expression *curse of dimensionality* is due to Bellman [109] and in statistics it relates to the fact that the convergence of any estimator to the true value of a smooth function defined on a space of high dimension is very slow. This curse is particularly a problem when solving the epistasis detection problem within a parametric paradigm, such as when adopting a regression framework.

Standard (automatic) stepwise procedures that are popular in regression-based model-building may also miss interactions that occur in the absence of detectable main effects [110]. In addition, regression-based approaches may not be optimal in identifying interactions when they are applied to rare variants because of the rare variants' low frequencies and weak signals [42, 111]. Hence, more advanced and efficient methods are needed to identify gene–gene interactions and epistatic patterns of susceptibility.

To date, several methods (beyond simple regression approaches) in epistasis screening methodology have been released [20], and several criteria have been used in an attempt to make a classification of the available approaches. Some of these criteria are: the strategy is exploratory in nature or not, modeling is the main aim or testing is, the epistatic effect is tested indirectly or directly, the approach is parametric or non-parametric, the strategy uses exhaustive search algorithms or takes a reduced set of input-data, that may be derived from prior expert knowledge or some filtering approaches. Shang et al. [112] based on Kilpatrick [113] identified thirty-six methods and classified them into three categories according to their search strategies. The classifications used as presented in Figure 1.2 are exhaustive search, stochastic search, and heuristic search.

Despite the fact that Shang et al. [112] classified several methods into a category based on a general search strategy, issues still remain on how to compare performances of these methods. Comparing epistatic methods (mainly done via simulation-based power studies) seems to be comparing "apples and oranges" because the comparison is not a direct comparison of key characteristics of the methods themselves but of a "total approach". For instance, BOOST of Wan et al. [114] and AntEpiSeeker of Wang et al. [115] both involve two-stages testing in order to determine whether the interactive effect of a SNP pair is significant. BOOST uses a likelihood ratio test in the first stage and a *chi*-squared test in the second stage. On the other hand, AntEpiSeeker, a two-stage ant colony optimization algorithm uses a c*hi*-squared test in the first stage and in the second stage it conducts an exhaustive search of interactions within the highly suspected SNP sets, and within the reduced set of SNPs with top ranking trait levels. When data consist of binary observations, the *score* statistic is the same as the chi-squared statistic in the Pearson's chi-squared test. Only when the sample size becomes large, the statistical power of the score test will be similar to that of the likelihood ratio test. Hence, the question of interest is to know how methods would compare when making them as alike as possible, in the sense of replacing test statistics by their (statistically) most optimal counterparts and using the same multiple testing correction strategy (when the nature of the method allows doing so). This requires adapting existing methods, methods for which the source code is neither always easily accessible nor readable.

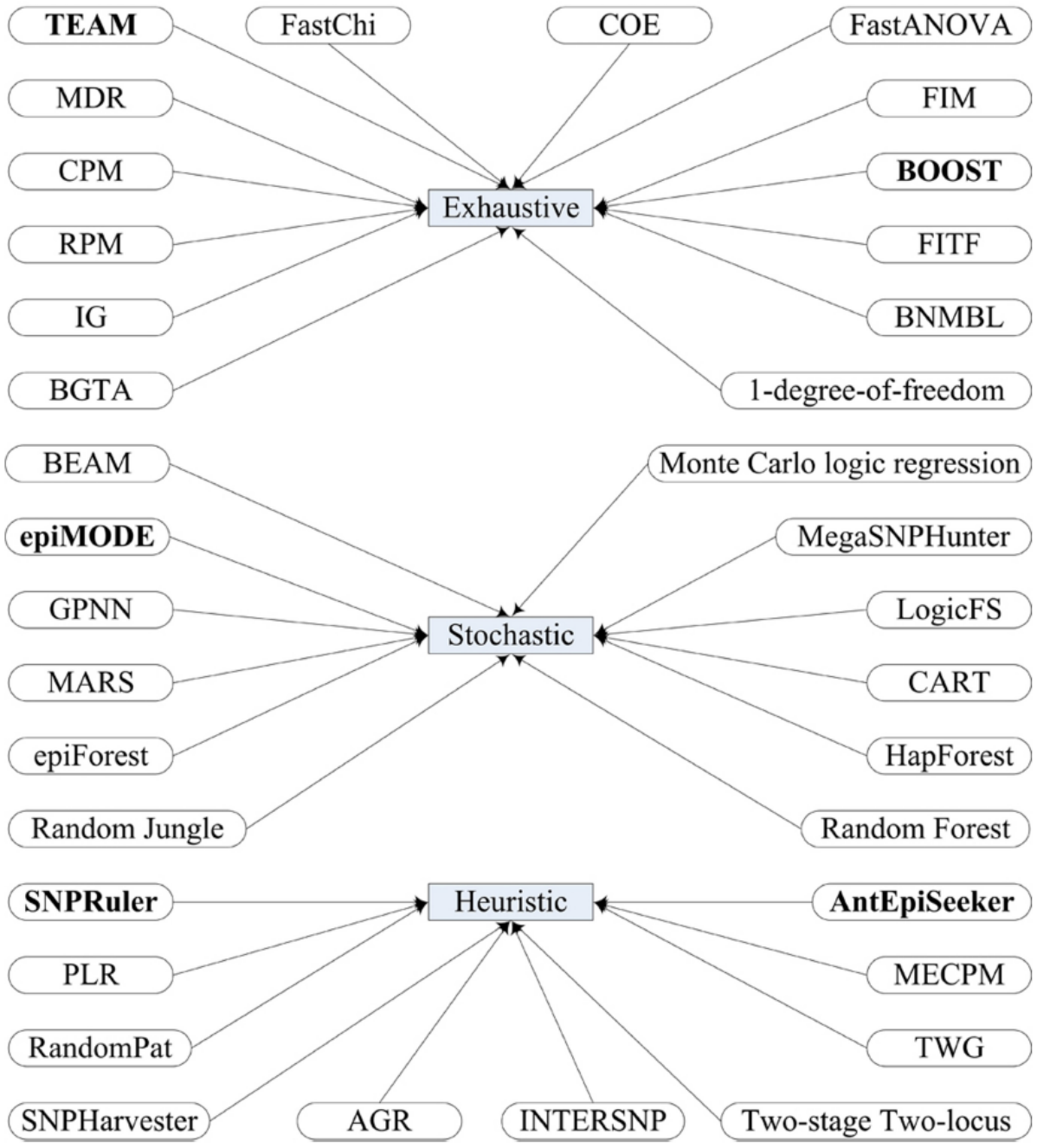

Figure 1. 2 Classification of the methods that detect epistasis.

Legend All methods can be classified into three categories according to their search strategies, i.e., exhaustive search, stochastic search, and heuristic search. Methods with bold names are described and evaluated in detail in the Source manuscript. *Source: Shang et al. [112]*.

Among the methods presented in Figure 1.2 is the Multifactor Dimensionality Reduction (MDR), a non-parametric method of Ritchie et al. [116]. MDR offers an alternative to traditional statistical methods such as logistic regression. The method is model-free and non-parametric in the sense that it does not assume any particular genetic model and that it does not estimate any parameters, respectively. MDR nicely tackles the dimensionality problem involved in interaction detection for binary traits by pooling multi-locus genotypes into two groups of risk based on some threshold value. Those cells with a case/control ratio equal to or above the threshold are labeled as *High*-risk and the remaining cells as *Low*-risk. The main steps of MDR are presented in Figure 1.3.

However, the first versions of the MDR method had some 'major' drawbacks including that some important interactions could be missed due to pooling too many cells together. For instance, in the case where there are cases but no controls, the cell is labeled *High* risk and when there are controls but no cases, the cell is labeled *Low* risk. It is only restricted to binary traits (case-control studies and discordant sib-pairs) with balanced designs (i.e., it strictly required each individual in the dataset to have observed data for each variable otherwise the program would crash). The method could not adjust for lower-order effects and confounding factors. In addition, an MDR analysis could only reveal at most one significant epistasis model with the selection based on computationally demanding cross-validation and permutation strategies. For each number of factors under consideration, the best model selected by MDR is the one with the lowest prediction error and maximum cross-validation consistency. The 10-fold cross-validation procedure may be repeated a number of times to reduce the possibility of poor estimates of the prediction error that are due to chance divisions of the data. In this case, selection criteria are averaged over runs.

The easy-to-use and well-documented MDR software supporting the MDR method and its initial successes in practice (e.g. [117-120]), stimulated researchers to look more closely into dimensionality reduction methodologies as a way to make progress in GWAI studies. The MDR community was born. MDR open source version is freely available via *www.epistasis.org* and *http://ritchielab.psu.edu.*

Since its conception, about 400 methodological and applied papers have emerged that build on or use multifactor dimensionality reduction principles. Table 1.1 shows a list of MDR-related (methodological) papers that were published since the first publication of MDR in 2001, till the time this thesis was submitted. In particular, Table 1.1 provides information about targeted study design (i.e. whether the method is applied to unrelated or related

individuals), the outcome type that the method was applied to, the ability to deal with population stratification, the ability to handle missingness or requires complete cases (requiring a priori imputation impute) and whether the method allows for covariates or confounders adjustment. The last column of Table 1.1 summarizes/discusses the key features of the variation to the initial MDR method. We remark that although some of the methods can be extended beyond what has currently been described for the study design, we limited our categorization to those situations explicitly discussed in the referenced research papers. While it 'might not be exhaustive', we tried our best to collect all available papers; the collection was obtained through PubMed searches (*www.ncbi.nlm.nih.gov/pubmed:* with "multifactor dimensionality reduction" in the abstract or main body of the manuscript) and the "epistasis blog" (*www.epistasis.org*). We also appended our own work (published or submitted papers, papers under construction and presented abstracts).

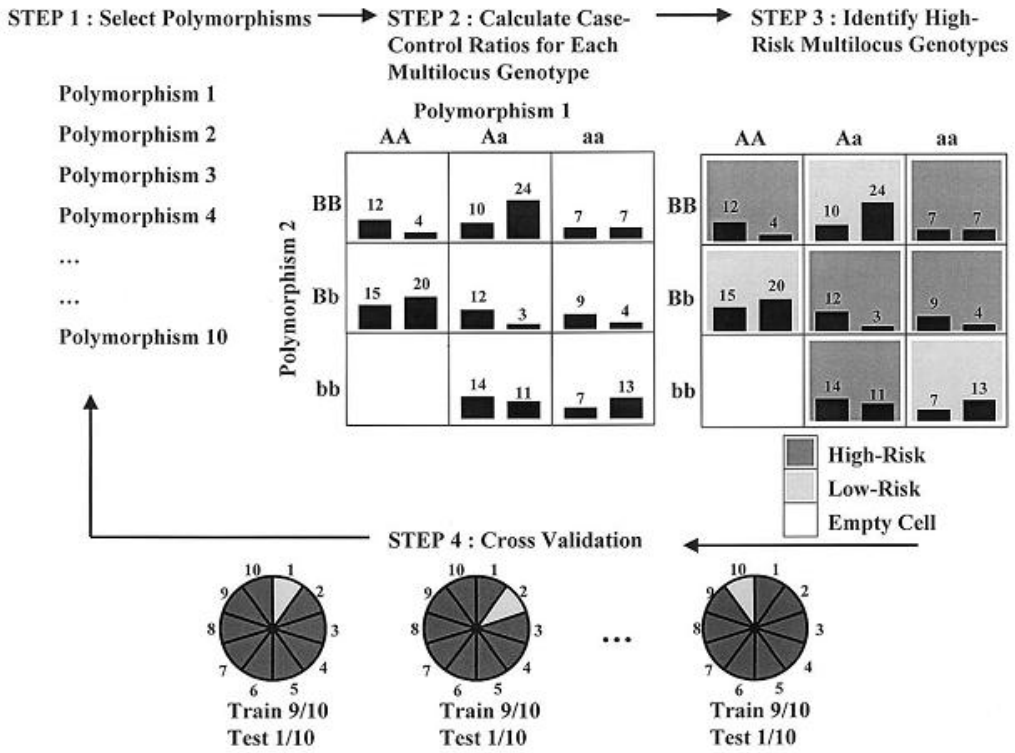

Figure 1. 3 Summary of steps involved in implementation of the MDR method.

Legend A set of *n* genetic and/or discrete environmental factors is selected; the *n* factors and their possible multifactor classes or cells are represented in *n*-dimensional space; each multifactor cell in *n*-dimensional space is labeled as either "*High*-risk" or "*Low*-risk"; and the prediction error of each model is estimated. For each multifactor combination, hypothetical distributions of cases (*left bars in boxes*) and of controls (*right bars in boxes*) are shown. *Source: Ritchie et al. [116]*.

Table 1.1 A list of MDR related methodological publications since the conception of the Multifactor Dimensionality Reduction method by Ritchie et al.[116].

Name	Study design[1]	outcome type[2]	Population stratification[3]	Missing genotypes allowed[4]	Covariates allowed[5]	Key references	Extensions to the original method
MDR	1	1	2	4	3	Ritchie et al. 2001 [116]	
MDR	1	1	2	3	3	Hahn et al. 2003 [121]	handles missing data by defining a new fourth level to be used when missing data is encountered, overcoming the need to only use complete data for analysis
EMDR	3	1	2	3	3	Mei et al. 2005 [122]	provides model selection without cross validation, and use a chi-square statistic as an alternative to prediction error.
MDR-PDT	3,4	1	2	3	3	Martin et al. 2006 [123]	handles families of diverse structure, overcoming the restriction of MDR to family data of discordant-sib-pairs or trios
MDR	1	1	2	3	3	Motsinger et al. 2006 [124]	reduces the number of MDR Cross validation intervals from ten to five hence reduced computation time
OR MDR	1	1	2	3	1	Chung et al. 2007 [125]	uses the odds ratio as a quantitative measure of disease risk, allowing one to quantify the measure of disease risk for each combination of genotypes. In addition it reduces false positives and negative errors when the ratio of cases to controls in a cell is equal to the preset threshold
MDR	1	1	2	3	3	Velez et al. 2007 [126]	accommodates imbalanced designs and utilizes balanced accuracy instead of accuracy, overcoming restriction to only balanced designs
GMDR	1,2	1,2	2	3	2	Lou et al. 2007 [127]	applicable to both dichotomous and continuous outcomes in population studies and permits adjustment for all forms of confounding factors, overcoming restriction to only binary traits with no adjustment of confounders
LM MDR	1	1	2	3	3	Lee et al. 2007 [128]	estimates frequencies for empty cells from a parsimonious log-linear model, improving the MDR in classifying sparse or empty cells
MDR-Phenomics	3	1	2	3	1	Mei et al. 2007 [129]	identifies genetic effects within triad families by integration of phenotypic variables into MDR, hereby controlling for genetic heterogeneity
MDR	1	1	2	3	3	Bush et al. 2008 [130]	replaces classification error with different contingency measures to score model quality in order to improve the ability of MDR to detect gene-gene interactions
PGMDR	3,4	1,2	2	3	2	Lou et al. 2008 [131]	accommodates family-based designs of diverse structure instead of only sib-pairs and trios, also accommodating continuous traits
MB-MDR	1	1	2	1	2	Calle et al. 2008a,2008b [132, 133]	R-based method that introduces 3 risk group categories to avoid over pooling of individuals to 2 groups, outputs more than 1 model, adjusts for confounding factors and uses simulation-based null distribution to test for multiple testing, performs multiple testing separately for the high-and low-risk groups and averages the respective corrected p-values to obtain an overall significance, uses *logistf* to address the issue of parameter separation
FAM-MDR	4,5	2	2	1	2	Cattaert et al. 2008[a], 2010 [134, 135]	extends the MB-MDR to family data with the familial structure is removed via a polygenic model using the GenABLE package and residuals are used as the new traits, adjusts for covariates on polygenic residuals in a regression step and uses the new residuals at the new traits for FAM-MDR modeling, adopts a permutation permutation-based strategy for multiple testing

MDR	1	1	2	3	3	Motsinger 2008 [136]	uses *n*-locus permutation test, a separate null distribution is created for each *n*-level of interaction rather than an omnibus permutation test where a single null distribution is generated from the best model of each of at least 1000 randomized datasets. The comparison analysis confirmed the need to stick to the omnibus permutation test since it controls false positives without limiting power.
MDR	1	1	2	3	3	Pattin et al. 2009 [137]	tests hypothesis using an extreme value distribution instead of permutation test , overcoming the computational burden while maintaining power
MB-MDR	1	1	2	1	2	Van Steen et al. 2010[a], 2011 [138, 139]	C++ based MB-MDR that adopts step-down maxT procedure to adjust for multiple testing instead of a simple permutation strategy, applied to noisy data on binary outcomes
MB-MDR	2	2	2	1	2	Mahachie John et al. 2010a[a], 2011 [140, 141]	extends C++ MB-MDR to noisy data on continuous outcomes with and without adjusting for functional SNPs. Adjustment of the functional SNPs is performed in a regression based model and the residuals are considered as new traits for MB-MDR
MB-MDR	2	2	2	1	2	Mahachie John et al. 2010b[a], 2012 [142, 143]	extends adjustment of SNPs to non-functional SNPs as well. Different strategies are used to adjust prior to C++ MB-MDR as well as "on the fly adjustment", where the main effects of the interaction pair under investigation are adjusted for while running MB-MDR
MB-MDR	2	4,5	2	1	3	Savenije et al. 2010[a] [144]	extends C++ MB-MDR to multiple continuous traits using the Hotelling's T^2 test to allocate risk groups. When the original outcome is discrete, multinomial logistic regression if first applied and residuals are used as new traits in MB-MDR
MB-MDR	1	1	2	1	2	Cattert et al. 2010[a] [145]	multi-locus genotype cells in C++ MB-MDR are allocated based on ranking procedures (e.g. ranked according to their case to control ratios and apply the Wilcoxon rank-sum test of association between disease status and these ratios) hereby improving allocation of cells to High- and Low-risk groups and performance of MB-MDR
MB-MDR	1,2	1,2	2	1	2	Urrea et al. 2010 [146]	R package for R-based MB-MDR. The package now incorporates continuous outcomes in addition to only binary as at conception
PWMDR	1	1	2	3	3	He et al. 2010 [147]	addresses the issue of sparse and empty cells in higher dimensional contingency table by scoring each pair-wise interaction of the genetic factors involved and combine the scores of all such pair wise interactions
MDR	1	1	2	3	1	Gui et al. 2010 [148]	extends MDR to adjustment of only discrete covariates, allows MDR to at least incorporate some confounders
SDR	1	3	2	3	3	Beretta et al. 2010 [149]	extends MDR to survival outcomes while estimating individual multi-locus cells survival functions in a non-parametric way via the Kaplan-Meier method hereby able to handle censored data too.
RMDR	1	1	2	3	3	Gui et al. 2011a [150]	extends MDR to 3 categories (High-, Low- and unknown risk- groups) while using the Fisher's Exact Test for determining whether specific genotype combinations should be included in the overall MDR. The "unknown risk group" is excluded when calculating the balanced accuracy of the MDR model.
Surv-MDR	1	3	2	3	2	Gui et al. 2011b [151]	extends MDR to survival outcomes. Instead of comparing the case–control ratio of each multi-locus genotype to a fixed threshold T, here log-rank test statistics are used to compare the survival distributions of each multi-locus genotype combination and its complement
MDR	1	1	2	3	1	Winham et al. 2011 [152]	Extends MDR to R software for R friendly users via *MDR* package
PedGMDR	4	1,2	2	3	2	Chen et al. [153]	builts a minimal sufficient statistic approach [154] into the GMDR framework, informative nonfounder generates a pair of statistics for transmitted and pseudo nontransmitted individuals, infers the nontransmitted genotypes of an individual to construct a control for each offspring, doubling the sample size

PedG-MDR II	4	1,2	2	3	2	Chen et al. 2011 [155]	calculates the statistics on the observed sample directly (undoubled), and evaluates their p values by constructing the empirical reference distributions on the basis of the sufficient statistic on a null distribution, hence theoretically halve the computing burden and memory requirement of PedGMDR
MDR-SP	1	1	1	3	2	Niu et al. 2011 [156]	extends MDR to structured populations, uses an association test that is robust to population stratification, instead of using the ratio of cases to controls by MDR, to divide the multi-marker genotypes into high- and low risk groups
SVM-based PGMDR	3,4	1,2	2	3	2	Fang et al. 2012 [157]	allows detection for the main and interaction effects from multiple genotype combinations in family data while accounting for confounding factors
MB-MDR GWAI Protocol	1,2,3,4,5	1,2,3,4,5	2	1,2	2	Gusareva et al. 2011[a] [158]	GWA epistasis screening to enhance MB-MDR analysis on large-scale data
MB-MDR	2	2	1	1,2	2	Gusareva et al. 2012a[a], 2012b [159, 160]	extends the MB-MDR to population structure which is removed via a polygenic model using the GenABLE package and residuals are used as the new traits in MB-MDR
MB-MDR	2	2	2	1,2	2	Van Lishout et al. 2012a [161]	MB-MDR software description with details on An Efficient Algorithm to Perform Multiple Testing in Epistasis Screening
MB-MDR	1	3	2	1,2	2	Van Lishout et al. 2012b[a*] [162]	extends MB-MDR to survival outcomes while using log-rank test to allocate risk groups
Filter-based MDR	1	1	2	3	3	Dai et al. 2012 [163]	involves global testing of p-values in conjunction with filtration process performed via ReliefF algorithm

Explanation coding:
[1]Subject design: 1) case-control, 2) other-unrelateds, 3) trios, 4) nuclear families, 5) extended pedigrees
[2]Outcome Type: 1) binary, 2) continuous, 3) survival, 4) multivariate, 5) discrete other than binary
[3]Population Stratification: 1) yes, 2) no
[4]Missing genotypes allowed: 1) available cases, 2) method procedure deletes missing genotypes and only uses complete cases, 3) other, 4) no
[5]Covariates allowed: 1) only categorical, 2) all form of covariates including SNP lower order effects, 3) no
[a]Presented abstract [a*]Abstract to be presented in December 2012

1.8.3.1 Model-Based Multifactor Dimensionality Reduction

Within the context of extending the MDR method of Ritchie et al. [116], Calle et al. [132] developed the Model-Based Multifactor dimensionality reduction (MB-MDR) method, initially also focusing on dichotomous traits, accommodating confounding factors. The principal difference between MDR and MB-MDR, applied to binary traits, is that MB-MDR merges multi-locus genotypes exhibiting some significant evidence of *H(igh)* or *L(ow)* risk, based on association testing, rather than on comparison with a threshold value. In addition, those multilocus genotypes that either show no evidence of association or have no sufficient sample size contribute to an additional MB-MDR category, that of 'no evidence'. The introduction of the third category avoids pooling too many multilocus genotype cells together or forcing either a high risk or low risk label on them. The MB-MDR method, which now incorporates continuous traits as well, was first made available via an R package *mbmdr* [146].

It should be noted that the original MB-MDR method, although overcoming the MDR weaknesses mentioned in Section 1.8.3, has some major shortcomings. Firstly, it outputs *p*-values corrected for multiple testing via a simulation-based null distribution based on minor allele frequencies (MAFs). This leads to different null distributions for interaction pairs with different MAF combinations. As a consequence, the multiple testing procedure remains a cumbersome component. Secondly, Wald test statistics are used to test for associations. The Wald test is an asymptotic test and it assumes infinite amount of data [164, 165]. This is not the case when one compares multilocus genotypes, small sample sizes tend to be involved. In practice, infinite amounts of recorded data are never available. Hence, the Wald test is unreliable to use with MB-MDR or dimensionality reduction methods in general since it tends to be biased when data are sparse [166]. Thirdly, two types of null-distribution adjusted *p*-values are obtained, one *p*-value for the *H*-labeled multilocus genotypes *(*tested versus the *L* risk genotypes*)* and one *p*-value for the *L*-labeled multilocus genotypes (tested versus the *H* risk genotypes), say P_H and P_L respectively. In order to correct for multiple aggregation of cells, the overall adjusted *p*-value for the interaction pair under investigation is then computed as the minimum of P_H and P_L. To note, the 'no evidence' for risk category is not used when computing the adjusted *p*-values which is a drawback since, for data that are already hard to acquire, not all samples were used in the process. Fourthly, MB-MDR uses R package *logistf* [167], Firth's bias reduced logistic regression approach with penalized profile likelihood based confidence intervals for parameter estimates [168]. The reason for its implementation is to

guard against near-to-complete data separation problems leading to parameter estimation difficulties. However, *logistf* only targets binary traits with link function *logit*. Accommodating other link functions in the binomial family requires the implementation of package *brglm* (bias reduction in generalized linear models) [169]. Supported link functions are *logit*, *probit*, *cloglog* and *cauchit*. Although *brglm* is currently implemented only for the *binomial* family, the upcoming version is expected to work with all families supported by generalized linear models [170].

Based on the aforementioned issues related to the first implementation of MB-MDR [132], our lab decided to shift gears. We kept the "core features" of the method and then used these as foundations to develop different "faces" of the MB-MDR methodology, hereby addressing possible complications that emerged from practical applications on the fly. The software supporting this new viewpoint is also called MB-MDR and was written in C++ [171]. Because of its importance, we summarize again the core features of MB-MDR: 1) dimensionality reduction via multilocus genotype cell-labeling, allowing for a variety of label choices, depending on the evidence to enhance or reduce disease risk/mean trait/survival outcome, 2) association testing-based model selection instead of cross-validation-based model selection, where association tests are also used to derive the cell labels, 3) flexible multiple testing correction based on what is known from the "multiple testing community".

In what follows, we explain the different steps involved in MB-MDR analyses in more detail. For a sufficiently frequent bi-allelic marker, there are 3 theoretically possible genotypes. Hence, 2 bi-allelic markers give rise to 9 multilocus cells. Each of the 9 multilocus genotype cells alternatively constitute group 1. The remaining 8 multilocus genotypes constitute group 2. The key MB-MDR steps, translated to analyze quantitative traits, using the default options of the MB-MDR software, are summarized in Figure 1.4. In MB-MDR step 1, a Student *t*-test at significance level 0.1 is used to compare the mean trait values in the 2 aforementioned groups of multi-locus genotypes. In step 2, the cell-based results of step 1 are used to label significant cells as *H(igh)* or *L(ow)* and non significant ones as 'no evidence', *O*. The sign of the Student's *t*-test statistic is used to distinguish between *H* and *L*: a positive (negative) sign refers to *H (L)*. The result is a dimensionality reduction from a categorical multilocus genetic explanatory variable with 9 factor levels, to a new categorical explanatory variable with factor levels (labels) *H*, *L* and *O*. A new association test is subsequently performed to assess the relation between the newly created construct and the quantitative trait, *Y*, of interest. In particular, we consider the maximum of two Student *t*-tests, one comparing the *H*-cells versus

{*L*,*O*}-cells and one comparing the *L*-cells versus {*H*,*O*}-cells. In step 3, the overall significance is assessed by adopting the permutation-based maxT correction of Westfall and Young [172] with 999 replicates. This criterion uses all observations for every interaction under investigation and is independent of the minor allele frequencies. Thus, instead of being model allele frequency specific as in the original MB-MDR, we make use of permutation data to generate a reference distribution while maintaining type I control. Part of the computational concerns related to the aforementioned permutation-based significance assessment of multiple epistasis hypothesis tests jointly, were elevated by an improved implementation of the algorithm [171].

The first evaluation of our method was on simulated binary data with and without noise by Cattaert et al. [139]. It showed that MB-MDR has increased power over MDR to identify gene–gene interactions for most considered genetic models, even in the presence of error sources. From a clinical point of view, binary outcomes may be preferred as they facilitate setting diagnostic criteria for disease and offer a simpler interpretation of common effect measures from statistical models (such as odds ratios and relative risks). These advantages come with some information-loss. From a statistical point of view, this loss of information implies that more human samples may be required to attain prespecified power levels [173]. In addition, since many diseases are not uniquely defined by a binary trait (e.g., there exist several non-categorical asthma-related phenotypes such as log IgE and sensitization level), the need to develop a continuous face of MB-MDR emerged. This is the subject of this thesis. Some of the challenges include dealing with a more refined/detailed phenotype, with potential outliers and a wide variety of underlying distributions, as well as fully exploiting this potential wealth of information in the search for gene-gene interactions. We remark that other faces of MB-MDR exist, which address censored traits, and multivariate traits as well. Their implementation in the MB-MDR software and their validation is ongoing. Although Figure 1.4 presents a scenario for 2-order interactions using bi-allelic genetic markers, the “core features” of MB-MDR naturally accommodate higher-order interactions and multi-allelic markers as well.

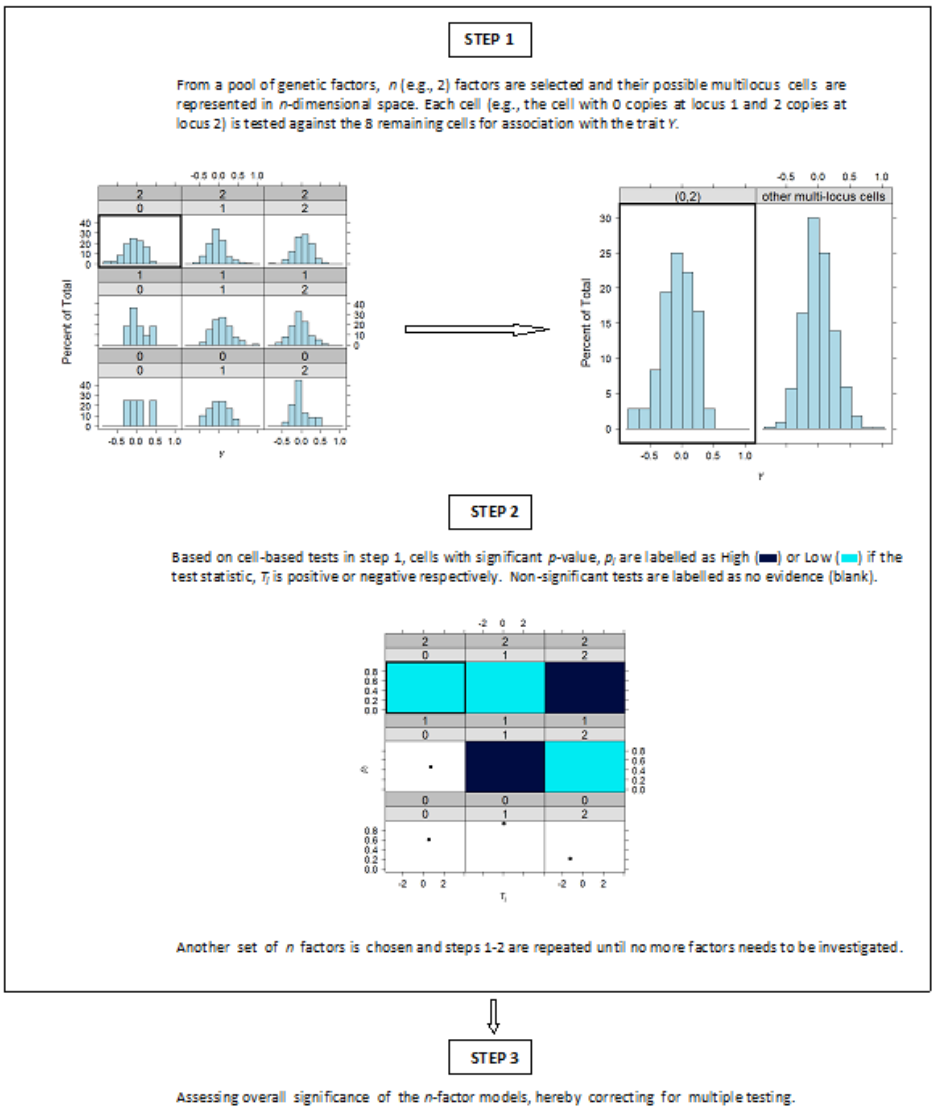

Figure 1. 4 Summary of the steps involved in MB-MDR analysis. *Source: Mahachie John et al.* [141, 143].

Chapter 2: Aims and Thesis Organization

2.1 Aims

The main aim of this thesis was to investigate the performance of the MB-MDR methodology adapted to quantitative traits, to identify the bottlenecks and to develop solutions accordingly. Validation of new developments was mainly done via simulated data on quantitative traits for unrelateds, using a variety of 2-locus epistasis models and scenarios'. Programs to simulate the data needed for our studies were written in R [174] and can be obtained upon request. In the validation assessment, special attention was given to power performance and false positive control properties. In addition to using simulated data, the ability to detect epistasis on real-life data (binary and quantitative traits) was investigated as well.

All MB-MDR based analyses were carried out with the C++ based MB-MDR software as introduced in Van Lishout et al. [171] and freely downloadable as an executable file from *http://www.statgen.ulg.ac.be*. Throughout this thesis, we will use MB-MDR interchangeably to indicate the "method" and the "software". Note that both method and C++ based software differ substantially from the MB-MDR method as developed by Calle et al. [175] (to date, accommodating both binary and continuous traits) and the associated R package "*mbmdr*" with its implementation, as outlined in the main introduction section of this thesis.

2.2 Thesis Organization

The work presented in this thesis can be divided into two components: a methodological development component and a practical application component. Having presented the general introduction in PART 1, we present the methodological aspects of the thesis in PART 2, consisting of 3 chapters. In chapter 1, we investigate the power of MB-MDR to detect gene–gene interactions in the absence or presence of error sources or noise (including genotyping errors, missing genotypes, phenotypic mixtures and genetic heterogeneity). In Chapter 2, we assess the performance of different corrective measures for lower-order genetic effects in MB-MDR epistasis detection. In Chapter 3, we evaluate the cumulative effect of deviations from normality and homoscedasticity on the overall performance of MB-MDR giving leads on the importance of adhering to general assumptions related to the implemented tests for association. We present practical applications in PART 3. In PART 4, we give a general discussion and future perspectives. Lastly, PART 5 presents a short curriculum vitae and the list of publications related to the author of this thesis.

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PART 2

METHODOLOGICAL DEVELOPMENTS

Chapter 1

Model-Based Multifactor Dimensionality Reduction to Detect Epistasis for Quantitative Traits in the Presence of Error-free and Noisy Data

Related publication

Jestinah M. Mahachie John, François Van Lishout, Kristel Van Steen (2011)

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Abstract

Detecting gene-gene interactions or epistasis in studies of human complex diseases is a big challenge in the area of epidemiology. To address this problem, several methods have been developed. One of these methods, Model-Based Multifactor Dimensionality Reduction, has so far mainly been applied to case-control studies. In this chapter, we evaluate the power of Model-Based Multifactor Dimensionality Reduction for quantitative traits to detect gene-gene interactions (epistasis) in the presence of error-free and noisy data. Considered sources of error are genotyping errors, missing genotypes, phenotypic mixtures and genetic heterogeneity. Our simulation study encompasses a variety of settings with varying minor allele frequencies and genetic variance for different epistasis models. On each simulated data, we have performed Model-Based Multifactor Dimensionality Reduction in two ways: with and without adjustment for main effects of (known) functional SNPs. In line with binary trait counterparts, our simulations show that the power is lowest in the presence of phenotypic mixture or genetic heterogeneity compared to scenarios with missing genotypes or genotyping errors. In addition, empirical power estimates reduce even further with main effects corrections, but at the same time, false positive percentages are reduced as well. In conclusion, phenotypic mixtures and genetic heterogeneity remain challenging for epistasis detection, and careful thought must be given to the way important lower-order effects are accounted for in the analysis.

1.1 Introduction

Understanding the effects of genes on the development of complex diseases and traits in humans is a major aim of genetic epidemiology. These kinds of diseases are controlled by complex molecular mechanisms characterized by the joint action of several genes which could have different effect sizes, from small to large. In this context, traditional methods within a regression paradigm involving single markers have limited use and more advanced and efficient methods are needed to identify gene-gene interactions and epistatic patterns of susceptibility. Over the past few years, data dimensionality reduction methods such as Multifactor Dimensionality Reduction (MDR) developed by Ritchie et al. [1] and introduced in PART 1 of this thesis, have gained popularity as epistasis screening tool and alternative to the more traditional methods.

Despite the fact that Luo et al. [2] in part recognized the necessity to adjust for covariates and to extend MDR to quantitative traits, issues related to significance assessment remain, as explained in detail by Cattaert et al. [3]. In addition, as much as geneticists try to avoid errors in the field of genetics, in reality, due to one reason or the other, data can be found to be associated with different error sources [4]. Given that the "core features" of the Model-Based Multifactor Dimensionality Reduction (MB-MDR) methodology, as explained in PART 1 (Section 1.8.3.1), naturally extend to quantitative traits, we evaluate the power of quantitative MB-MDR [5] to detect 2-locus gene-gene interactions with bi-allelic SNPs, for data affected or not affected by different error sources.

1.2 Materials and Methods

1.2.1 Introducing noise

Apart from simulating error-free data, we also simulate different error-sources to investigate their impact on the performance of MB-MDR. These involve introducing 5 and 10% missing genotypes (MG5 and MG10), 5 and 10% genotyping error (GE5 and GE10), 25 and 50% phenotypic mixtures (PM25 and PM50) and 50% genetic heterogeneity (GH). It is important to realize that the foregoing derivations of variance decomposition relate to a population as whole. When generating sources of error, estimates of variability will no longer tend to the estimates at the population level. In other words, the actual genotypic variance will no longer equal the assumed genetic variance. Missing genotypes (MG5 and MG10) and genotyping errors (GE5 and GE10) are also introduced in the null data, leading to a total of 255 simulation settings, so as to be able to assess the impact of these on MB-MDR's type I error control in the presence of noise.

In particular, scenarios MG5 and MG10 are generated by selecting genotypes completely at random from the original data and by setting them to missing. This introduces different per-individual and per-SNP percentages of missingness, reducing the effective sample size, yet maintaining the validity of the variance components estimates.

As in Ritchie et al. [6] genotyping error is simulated using a directed-error model of Akey et al. [7]. This model postulates that there is a larger probability for the minor allele to be consistently mis-genotyped (over-represented). In this study, either 5% (GE5) or 10% (GE10) of the available genotypes in the original data set are sampled. From these, homozygous genotypes for the common allele become heterozygous and heterozygous genotypes for the rare allele become homozygous. The effect of adding genotyping errors to the original data is that the actual genetic contribution σ^2_{gen} to the trait variance is reduced compared to the assumed genetic variance σ^2_g, of the simulation setting due to the additional variability (noise) introduced into the system (Table 1.1).

Table 1.1 Proportion $\sigma^2_{gen}/\sigma^2_g$ of the total genetic variance in error-free data that is due to genetics in the error-prone data, exhibiting either 5% (GE5) or 10% (GE10) genotyping errors, or 25% (PM25) or 50% (PM50) phenotypic mixture.

Model	***p***	**GE**		**PM**	
		5%	**10%**	**25%**	**50%**
M27	0.1	0.673	0.494	0.563	0.250
	0.25	0.857	0.742	0.563	0.250
	0.5	0.926	0.858	0.563	0.250
M170	0.1	0.667	0.489	0.563	0.250
	0.25	0.701	0.507	0.563	0.250
	0.5	0.740	0.546	0.563	0.250

Genetic heterogeneity is simulated such that there are actually two different two-locus combinations increasing/decreasing the phenotypic mean. Half of the individuals have one pair of functional SNPs (SNP1 and SNP2), and the other half have the other pair of functional SNPs (SNP3 and SNP4). Introducing the notations G_L (G_H) as the multi-locus genotypes leading to a Low (High) phenotypic mean, traits are simulated according to the following specified distributions:

$$Y|_{g\in G_L, g'\in G_L} \sim N(\mu_L, \sigma^2_{env}), \qquad Y|_{g\in G_L, g'\in G_H} \sim 0.5N(\mu_L, \sigma^2_{env}) + 0.5N(\mu_H, \sigma^2_{env}),$$
$$Y|_{g\in G_H, g'\in G_H} \sim N(\mu_H, \sigma^2_{env}), \qquad Y|_{g\in G_H, g'\in G_L} \sim 0.5N(\mu_H, \sigma^2_{env}) + 0.5N(\mu_L, \sigma^2_{env})$$

Minor allele frequencies of all 4 functional SNPs are taken to be equal, i.e., $p \in \{0.1, 0.25, 0.5\}$.

Phenotypic mixing in genetics may occur when a percentage of individuals with high phenotypic mean have genotype combinations that are consistent with low phenotypic mean. In particular, a mixing proportion of $w \in [0,1]$ of phenotypic mixture, trait values are simulated according to

$$Y|_{g\in G_L} \sim (1-w\pi)N(\mu_L, \sigma^2_{env}) + w\pi N(\mu_H, \sigma^2_{env}),$$
$$Y|_{g\in G_H} \sim w(1-\pi)N(\mu_L, \sigma^2_{env}) + [1-w(1-\pi)]N(\mu_H, \sigma^2_{env})$$

with mixing proportion either 25% (PM25) or 50% (PM50), and π, the probability of a multi-locus genotype giving rise to a high phenotypic mean μ_H.

1.2.2 Adjustment for main effects

In epistasis screening, some interactions can be identified simply because of highly significant lower-order effects, and are therefore not genuine. That is why we also in this chapter consider MB-MDR adjusted analyses in the following way: with and without adjustment for main effects of functional SNPs. Main effects are adjusted for in MB-MDR by first regressing them out in a data preparation step and then considering the residuals from the regression model as new traits. Two extreme ways of correcting are considered: the additive model and the codominant model. When adjusting for main effects in the presence of genetic heterogeneity, we take into account that different functional pairs are relevant for heterogeneous sub-populations.

1.2.3 Data Simulation

Data are simulated with 500 replicates in each simulation setting. Each replicate consists of 1500 unrelated individuals and 10 SNPs, 2 of which are functional. The minor allele frequencies of functional SNPs (SNP_1, SNP_2) are taken to be equal, and varying as $(p_1, p_2) = (p, p), p \in \{0.1, 0.25, 0.5\}$, whereas the minor allele frequencies of a non-functional marker SNP_j are fixed at $p_j = 0.1 + (j - 3) * 0.05, j = 3, \ldots, 10$. All SNPs are assumed to be in Hardy-Weinberg Equilibrium and in linkage equilibrium.

Two epistasis models that incorporate varying degrees of epistasis are considered: Model 27 and Model 170 of Evans et al. [8], hereafter referred to as M27 and M170 respectively. The models can be illustrated as follows:

$$M27 \approx \begin{pmatrix} 0 & 0 & 0 \\ 0 & 1 & 1 \\ 0 & 1 & 1 \end{pmatrix}, \qquad M170 \approx \begin{pmatrix} 0 & 1 & 0 \\ 1 & 0 & 1 \\ 0 & 1 & 0 \end{pmatrix}$$

Each row indexes the genotype at the first locus and each column refers to the genotype at the second locus. The number in each cell is the phenotypic mean for that genotype. In order to increase the phenotypic mean, M27 requires an individual to have at least one copy of the increaser allele at both loci whereas M170 requires an individual to be heterozygous at one locus and homozygous at the other. As *p* increases, the contribution to the total genetic variance of epistasis variance relative to main effects variances increases for M170 (decreases for M27) (Cattaert et al. [3] - Table 1). The phenotypic means for these epistasis models only

take two values, μ_L (Low phenotypic mean) and μ_H (High phenotypic mean). The total phenotypic variance σ^2_{tot}, i.e. the sum of genetic variance at both loci $2\sigma^2_1 = \sigma^2_{main}$ (the minor allele frequencies for the functional SNPs are taken to be the same), epistasis variance σ^2_{epi}, and environmental variance σ^2_{env}, is fixed at 1. As a consequence, the total genetic variance, defined as $2\sigma^2_1 + \sigma^2_{epi}$, has an interpretation of a broad heritability measure. Throughout this thesis, it will further be referred to as σ^2_g, to clearly indicate that the interpretation as a heritability is due to the imposed normalization constraints. The parameter σ^2_g is varied as $\sigma^2_g \in \{0.01, 0.02, 0.03, 0.05, 0.1\}$. Explicit formulae for these variance components can be obtained from Evans et al. [8].

In addition, 1000 null data sets are generated under the most general null hypothesis of no association between any of the 10 SNPs and the trait (i.e., $\sigma^2_g = 0$, no main effects and no epistasis).

1.3 Results

1.3.1 The impact of not correcting for lower-order effects

Table 1.2 gives an overview of MB-MDR empirical type I error rates in the presence and absence of noise (MG and GE). We observe that MB-MDR empirical type I error percentages are close to the nominal type I error percentage of 5%, when no correction for main effects is performed. When we adjust for main effects, type I error percentages are further reduced and seem to drop below the theoretical value. Similar trends are observed when genotyping errors and missing genotypes are introduced in the data.

Table 1. 2 Type I error percentages for data generated under the general null hypothesis of no genetic association in the absence and presence of noise.

			Main Effects Correction	
p	**Noisiness**	**No Correction**	**Additive**	**Codominant**
0.1	None	0.055	0.055	0.049
0.25		0.051	0.038	0.036
0.5		0.054	0.039	0.030
0.1	MG5	0.046	0.039	0.038
0.25		0.051	0.034	0.036
0.5		0.052	0.044	0.047
0.1	MG10	0.046	0.041	0.041
0.25		0.054	0.043	0.043
0.5		0.048	0.045	0.038
0.1	GE5	0.051	0.037	0.037
0.25		0.048	0.038	0.036
0.5		0.038	0.035	0.031
0.1	GE10	0.049	0.037	0.032
0.25		0.049	0.033	0.031
0.5		0.054	0.039	0.039

MG5 (MG10) = 5% (10%) missing genotypes and GE5 (GE10) =5% (10%) genotyping errors

Power estimates of MB-MDR to detect the correct interacting pair, SNP1 x SNP2 (in the absence of genetic heterogeneity) from error-free and noisy data are shown in Figure 1.1. The actual numerical results of the power profiles plotted in Figure 1.1 are presented in Appendix Table A1. This table also includes the corresponding empirical power estimates related to main effects adjusted analyses.

In the absence of any adjustment for lower order genetic effects (i.e., main effects), we notice that power profiles largely follow the same trajectory, except in the presence of 50% phenotypic mixture (PM50). For all scenarios of *p*, power increases with increasing σ_g^2 (Figure 1.1 and Table A1). Moreover, the power of MB-MDR (ranging from 54% to 100%, *p*=0.1, 38% to 100%, *p*=0.25, 33% to 100%, *p*=0.5 under M170 and from 44% to 100%, *p*=0.1, 43% to 100%, *p*=0.25, 39% to 100%, *p*=0.5 under M27 for error-free data; Table A1) is hardly affected by introducing small percentages of missing genotypes (MG5 in Figure 1.1), irrespective of the epistasis model under investigation. Power estimates for MG5 range from 42% to 100%, *p*=0.1, 33% to 100%, *p*=0.25, 28% to 100%, *p*=0.5 and from 33% to 100%, *p*=0.1, 34% to 100%, *p*=0.25, 31% to 100%, *p*=0.5 under M170 and M27, respectively (Table A1). For MG10, power obviously reduces further, but not in a dramatic way compared to MG5: power estimates reduce to a minimum of 31%, *p*=0.1, 25%, *p*=0.25, 25%, *p*=0.5 and to a minimum of 31%, *p*=0.1, 28%, *p*=0.25, 22%, *p*=0.5 for M170 and M27, respectively).

When 5% genotyping errors are introduced in the population, systematically lower power curves are obtained than in the presence of randomly missing genotypes. However, high percentages of genotyping error (GE10) or high percentages of phenotypic mixture (PM50) generally lead to the lowest power performance of MB-MDR (Figure 1.1). For model M170 power estimates in the presence of 10% genotyping errors are in the range of 12% to 100%, *p*=0.1, 8% to 100%, *p*=0.25, 12% to 100%, *p*=0.5 and in the range of 9% to 100%, *p*=0.1, 20% to 100%, *p*=0.25, 26% to 100% , *p*=0.5 for model M27 (Table A1). High percentages of phenotypic mixture have a negative impact on MB-MDR power, which is also indicated by the minimally observed empirical power estimates for PM50. Power estimates for the latter are in the range of 3% to 98%, *p*=0.1, 3% to 97%, *p*=0.25, 2% to 95%, p=0.5 for M170 and in the range of 3% to 95%, *p*=0.1, 2% to 97%, *p*=0.25, 3% to 95%, *p*=0.5 for M27.

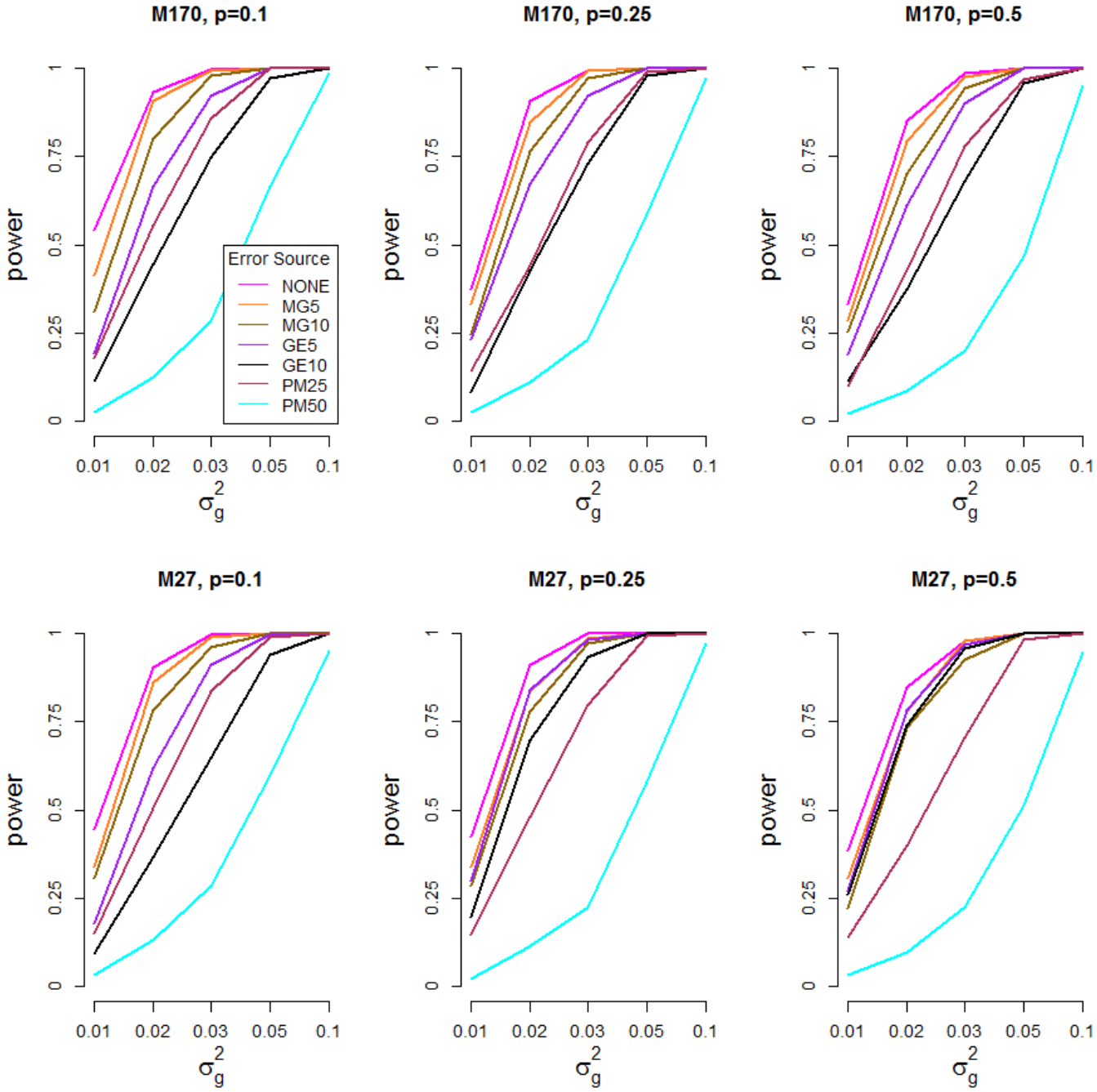

Figure 1. 1 Empirical power estimates of MB-MDR as the percentage of analyses where the correct interaction (SNP1 x SNP2) is significant at the 5% level, for error-free and noise-induced simulation settings.

Legend Results are shown for MB-MDR analysis without main effects adjustment and simulated scenarios other than GH.

Not surprisingly, there is a higher chance of identifying epistasis models for analyses without main effects correction as compared to analyses that do account for lower-order effects. The latter epistasis models usually involve other SNPs pairing with one of the functional SNPs (results not shown) and should therefore be considered as false positives. Empirically estimated false positive percentages, for a variety of scenarios, excluding GH settings, are reported in Table A2 ("No Correction" versus "Main Effects Correction" estimates). For error-free data, and no adjustments for main effects, the false positive percentage of MB-MDR of identifying a significant epistasis model not involving the actual functional pair of SNPs ranges from 28% to 100%, p=0.1, 6% to 53%, p=0.25, 6% to 7%, p=0.5 for M170 and from 15% to 99%, p=0.1, 26% to 100%, p=0.25, 38% to 100%, p=0.5 for M27. When main effects are accounted for in error-free data, the false positive percentage ranges from 3% to 39%, p=0.1, 3% to 12%, p=0.25, 3% to 6%, p=0.5 under M170 and from 3% to 7%, p=0.1, 3% to 21%, p=0.25, 2% to 98%, p=0.5 under M27 (Table A2). In general, Table A2 shows that irrespective of how the main effects adjustment is performed (using an additive or codominant model) and irrespective of the type of noisiness introduced, false positive percentages are typically lower than their "uncorrected" counterparts.

1.3.2 The impact of appropriately correcting an epistasis analysis for lower-order effects

Profiles for the empirical power estimates of MB-MDR to detect the correct two functional loci from error-free data with (additive and codominant) main effects correction and without main effects adjustment are plotted in Figure 1.2. Here, we observe that the power to identify the correct causal pair is reduced when a main effects correction is performed, with the lowest power levels obtained for codominant correction. The discrepancy between additive and codominant main effects adjustment is particularly pronounced for M27 and p=0.5. For M170 and p=0.5, the nature of the lower-order effects adjustment has virtually no influence on power. Power profiles for different sources of noise, according to main effects adjustment method, are given in Figure A1-i (missing genotypes), Figure A1-ii (genotyping errors) and Figure A1-iii (phenotypic mixture). The empirical power estimates used to generate Figures A1 are also presented in Table A1. Here, drawing conclusions is more subtle, although generally speaking, empirical power estimates are smaller with codominant correction as opposed to additive correction.

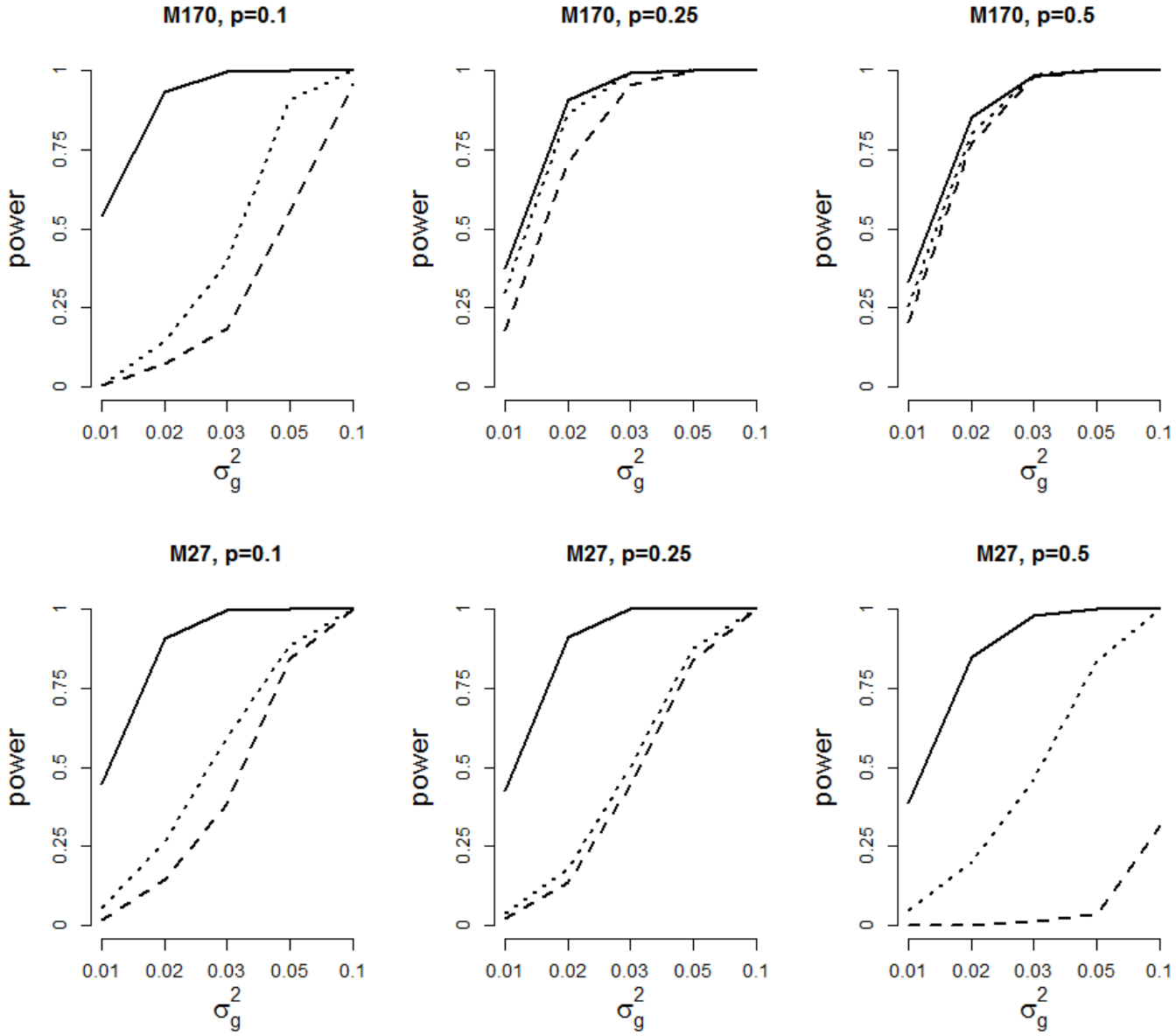

Figure 1. 2 Empirical power estimates of MB-MDR as the percentage of analyses where the correct interaction (SNP1 x SNP2) is significant at the 5% level, for error-free simulation settings.

Legend No main effects adjustment (—), main effects adjustment via additive coding (…), and codominant coding (---).

Numerical values on the effect of using different main effects adjustments on the false positive percentage to identify incorrect two-locus models can be derived from Table A2. For error-free data, the false positive percentages after additive main effects correction range from 5% to 39%, *p*=0.1, 5% to 12%, *p*=0.25, 3% to 6%, *p*=0.5 for M170 and from 4% to 7%, *p*=0.1, 4% to 21%, *p*=0.25, 9% to 98%, *p*=0.5 for M27. Using codominant coding to adjust for lower order effects, the false positive percentages range from 3% to 6%, *p*=0.1, 3% to 4%, *p*=0.25 or *p*=0.5 for M170 and from 3% to 6%, *p*=0.1, 3% to 3%, *p*=0.25 and from 2% to 4%, *p*=0.5 for M27. In fact, the practice of correcting an MB-MDR epistasis analysis using a codominant main effects model has the tendency to be over-conservative (Table A2).

Genetic heterogeneity

So far, we have not yet discussed the performance of MB-MDR for quantitative traits in the presence of genetic heterogeneity. Figure 1.3 shows empirical power curves to identify true genetic interactions in the presence of GH in a variety of simulation settings. Results are shown for MB-MDR analysis without main effects correction (Figure 1.3, row 1 for M170 and row 3 for M27) and with main effects correction (additive coding) adjustment (Figure 1.3, row 2 for M170 and row 4 for M27). As in non-GH settings, power estimates are larger when no correction for main effects is performed than when main effects are accounted for, with generally the most severe power loss observed for codominant main effects correction.

However, when the contribution of main effects to the total genetic variance is ignored, false positive percentages rise as well, ranging from 7% to 100% for M27 and from 4% to 97% for M170. When we adjust for main effects (additive coding), power estimates to identify the first pair (SNP1 x SNP2) drop to less than 50% for both M27 and M170, with the exception of M170. For the latter, and a genetic variance of 0.1, MB-MDR power is estimated to be 95% and 92% for *p*=0.25 and 0.5, respectively. Under a codominant correction, power estimates drop to less than 7% for both models with the exception of *p*=0.25 or 0.5 and σ_g^2 = 0.05 or 0.1. For the latter, power is estimated to be 15% and 26% for M170 and M27, respectively when *p*=0.1 and σ_g^2 =0.1. For M27, power=31%, *p*=0.25 and σ_g^2 =0.1. For M170, *p*=0.25 or 0.5, power estimates are around 30% and 88% for σ_g^2 =0.05 and 0.1, respectively. Detailed information about empirical power estimates are given in Table A7.

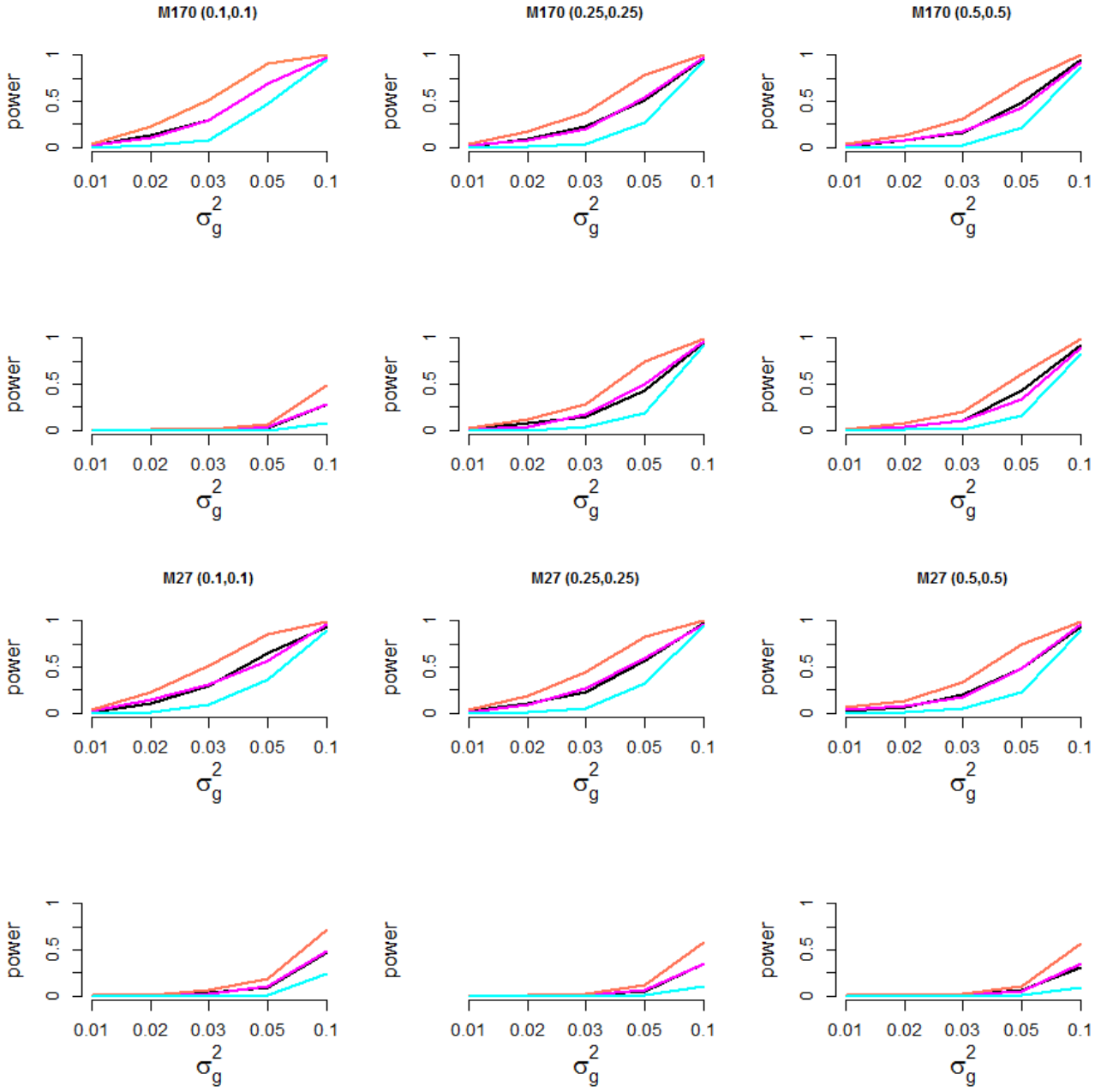

Figure 1. 3 Empirical power estimates of MB-MDR as the percentage of analyses where the correct interactions (SNP1 x SNP2) and/or (SNP3 x SNP4) are significant at the 5% level, in the presence GH.

Legend First 2 rows: MB-MDR without and with main effects correction for model M170 respectively. Last 2 rows: MB-MDR without and with main effects correction for model M27 respectively. Main effects are corrected for via additive coding. Different definitions for power are adopted: power to identify both interacting pairs SNP1 x SNP2 and SNP3 x SNP4 (cyan); power to identify SNP1 x SNP2 (black); power to identify SNP3 x SNP4 (magenta), power to identify at least one of the interactive pairs (coral).

1.4 Discussion

Understanding the effects of genes on the development of complex diseases is a major aim of genetic epidemiology. Several studies have indicated that MDR has good power to identify gene-gene interactions in both simulated and real-life data [6]. Although MB-MDR has profiled itself as a promising extension of MDR accommodating study designs that are more complex than unrelated case-control settings [3, 9-11], a thorough investigation of its full potential, under a variety of real-life distorting factors, such as missing genotypes, genotyping errors, phenotypic mixtures and last but not least genetic heterogeneity, has never been carried out in the context of quantitative traits.

This study has evaluated the power of MB-MDR for quantitative traits and unrelated individuals, in identifying gene-gene interactions for two different epistasis models. Scenarios with and without noisy data, as well as epistasis screening with and without lower-order effects adjustments have been considered. Although our simulations only involved 10 SNPS, conclusions about observed patterns largely remain the same when increasing the number of genetic markers (e.g. empirical power results for non-GH settings without main effects adjustment, Figure A2). Note that, an increasing number of SNPs will lead to an increasing number of interacting pairs, resulting in an elevated multiple testing burden, and hence resulting in reduced power.

A first important finding is that MB-MDR adequately deals with one of the most major concerns in genetic association analysis studies (especially those targeting higher-order gene-gene interactions), namely avoiding that the overall type I rate is out of control (Table 1.2). The apparent slightly conservative results obtained when MB-MDR screening explicitly accounts for lower-order main effects, are not surprising. Indeed, under the general null hypothesis of no genetic association, adjusting for main effects involves over-fitting and hence unnecessary over-correction. However, all the empirical estimates of the MB-MDR type I error rate in Table 1.2 fall within the interval [0.025,0.075], satisfying Bradley's [12] liberal criterion of robustness. This criterion requires that the type I error rates are controlled for any level α of significance, if the empirical type I error rate $\hat{\alpha}$ is contained in the interval $0.5\alpha \le \hat{\alpha} \le 1.5\alpha$. Note that we remark that, since MB-MDR assesses global significance using resampling-based maxT adjusted p-values, the FWER will always be weakly controlled at 5%, provided the assumptions of the Westfall and Young approach [13] are attained.

A second important finding is that MB-MDR's power performance under different scenarios can be largely explained by the quantification of the actual genetic variance σ^2_{gen} and by the decomposition of the total genetic variance into contributions of main effects and epistasis, and/or by the decomposition of main effects into additive and dominance variance. Empirical decompositions based on classical variance components analysis of Sham [14], are reported in Tables A3 for M170 and A4 for M27 in the absence of GH, and in Tables A5 in the presence of GH. Each of these estimates is based on simulation setting's sample size (750 000 individuals). These results support our theoretically derived variance components, which are summarized in Table A6.

In particular, the observed lowest power performances of non-GH settings for GE10 and PM50 can be explained by the fact that over-representation of the minor allele as well as introducing phenotypic mixture result in a loss of actual genetic variance (Table 1.1) and therefore a loss of power. The theoretical results, indicating that a 50% reduction in total genetic variability is established when 50% phenotypic mixture is introduced in error-free data (Table 1.1) are supported by our empirical results (e.g., Tables A3 for M170 and A4 for M27, comparing σ^2_{gen} with σ^2_g.

When 50% genetic heterogeneity is present, theory supports our empirical results in that the total actual genetic variance due to the two causal pairs of markers is twice the total actual genetic variance due to a single pair (Tables A5). Moreover, since we have introduced two possible genetic routes for an individual to be genetically predisposed for the trait of interest under GH (route 1 via SNP1 x SNP2 or route 2 via SNP3 x SNP4), the actual genetic variance in the pooled data will be half the genetic variance in the error-free data (see also Tables A5-ii for M170 and A5-iv for M27). The total genetic variance due to a single causal pair approximates $\sigma^2_g/4$ (Table A5-i and A5-iii), which is due to the fact that the 2 pairs have the same minor allele frequencies. Therefore, the theoretical genetic variance is split between the two pairs and thereafter between the 2 SNPs. MB-MDR was shown to be rather robust in the presence of missing genotypes and genotyping error. Note that MB-MDR that MB-MDR handles missing genotypes by using all available cases for the SNP pair under investigation. Hence, no individuals with missing data are a priori removed from the analysis, except when functional SNPs that are adjusted for in regression models have (partially) missing information.

A third finding is that accounting for important lower-order genetic effects in epistasis screening should be made standard. There is a debate about how to best model and test for both main effects and interactions or for interactions only when epistasis is present [15]. Although a fully non-parametric screening approach (e.g., such as MDR) is beautiful in that it does not require specifying particular genetic models, there is still a need to adjust for lower-order genetic effects via a parametric paradigm when targeting significant gene-gene interaction models. The Model-Based MDR (MB-MDR) offers a flexible framework to make these adjustments. For MDR-like applications other than MB-MDR this is far from obvious. For instance, MDR for binary traits, Ritchie et al. [6] does not accommodate taking corrective measures for lower order effects. Although significant main effects can be filtered out prior to an MDR screening, this happens at the cost of missing out on genuinely true interactions.

Furthermore, examining the decomposition of the total genetic variance has shed more light on the scenarios in which an adjusted MB-MDR analysis is warranted. For instance, when the minor allele frequency of the causal loci is 0.5, model M170 is a pure epistatic model (Table A3: empirical estimates $\sigma^2_{epi}/\sigma^2_{gen}$ approximate 1). Hence in this scenario the effects of correcting for main effects are taken to the extreme. Clearly, any correction for lower-order effects would be an over-correction. On the other hand, since there is no true evidence for main effects in this model, any adjustment for main effects will only remove a small portion of the variability (Table A3: M170, p=0.5; empirical estimates of $\sigma^2_{main}/\sigma^2_{gen}$ are close to zero), resulting in false positives for the corrective analysis that are similar to those for the un-corrective analysis (Table S2: M170, p=0.5; empirical estimates close to 5% also when not adjusting for main effects). In effect, the contribution of main effects becomes increasingly important with increasing p for M27 (≈32%, p=0.1, ≈61%, p=0.25 and ≈85%, p=0.5) and the reverse holds for M170 (≈59%, p=0.1, ≈11%, p=0.25 and ≈0%, p=0.5) (Table A3, A4 and Table A6).

For model M170 and GH scenarios involving p either 0.25 or 0.5 for the causal pairs, the epistatic variance explains a relatively large proportion of the total genetic variance in the data ($\sigma^2_{epi}/\sigma^2_{gen}$ >87%; Table A5-ii), and correcting for main effects therefore has little effect on power. In contrast, for Model M170 and p=0.1 for the causal pairs, main effects do make an important contribution to the total genetic variance ($\sigma^2_{main}/\sigma^2_{gen}$ > 57%; Table A5-i and Table A5-ii) compared to epistasis effects, which translates into a severe empirical power loss and

power is dramatically reduced when proper accountancy for lower order effects is being made (Figure 1.3).

Summarizing, dealing with phenotypic mixtures and genetic heterogeneity will remain challenging for epistasis screening methods, for some time to come. Our empirical results suggest that more work is needed to better accommodate these particularities. Benefits may be gained from identifying the trait-specific factors (genetic or non-genetic) that best characterize mixed phenotypic populations. For genetic heterogeneity, the genes in which the loci are present can be part of different etiological pathways leading to the same disease or be part of the same pathway. According to Heidema et al. [16], irrespective of the biological mechanism that gives rise to genetic heterogeneity, the association of the loci with the disease will be reduced if the total sample is used for measuring the association, as was done in this study. A method that is not robust in the presence of genetic heterogeneity will most likely suffer from a decrease in power to detect genetic effects. As our main effects corrective analyses have suggested, a way forward may be to use methods to identify the latent classes and to adapt the epistasis screening accordingly.

Finally, any epistasis screening should properly account for lower-order effects in order to be able to claim that an identified interaction involves a significant epistatic contribution to the total genetic variance.

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Chapter 2

Lower-order Effects Adjustment in Quantitative Traits Model-Based Multifactor Dimensionality Reduction

Related publication

Jestinah M. Mahachie John, Tom Cattaert, François Van Lishout, Elena S. Gusareva, Kristel Van Steen (2012)

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Abstract

Identifying gene-gene interactions or gene-environment interactions in studies of human complex diseases remains a big challenge in genetic epidemiology. An additional challenge, often forgotten, is to account for important lower-order genetic effects during this process. These may hamper the identification of genuine epistasis. If lower-order genetic effects contribute to the genetic variance of a trait, identified statistical interactions may simply be due to a signal boost of these effects. Via simulations, we assess the performance of different corrective measures for lower-order genetic effects in Model-Based Multifactor Dimensionality Reduction epistasis detection, using additive and codominant coding schemes. Performance is evaluated in terms of power and family-wise error rate. Our simulations indicate that empirical power estimates are reduced with correction of lower-order effects, likewise family-wise error rates. Easy-to-use automatic SNP selection procedures, SNP selection based on "top" findings, or SNP selection based on *p*-value criterion for interesting main effects result in reduced power but also almost zero false positive rates. Always accounting for main effects in the SNP-SNP pair under investigation during Model-Based Multifactor Dimensionality Reduction analysis adequately controls false positive epistasis findings. This is particularly true when adopting a codominant corrective coding scheme. In conclusion, automatic search procedures to identify lower-order effects to correct for during epistasis screening should be avoided. The same is true for procedures that adjust for lower-order effects prior to Model-Based Multifactor Dimensionality Reduction and involve using residuals as the new trait. We advocate using "on-the-fly" lower-order effects adjusting when screening for SNP-SNP interactions using Model-Based Multifactor Dimensionality Reduction analysis.

2.1 Introduction

Complex diseases commonly occur in a population and are a major source of discomfort, disability and death worldwide. They are believed to arise from multiple predisposing factors, both genetic and non-genetic, each factor potentially having a modifying effect on the other. Detecting gene-gene interactions or epistasis in studies of human complex diseases is a big challenge in genetic epidemiology. An additional challenge is to account for important lower-order genetic effects in order to reduce false positive epistasis results.

To date, several strategies are available, within the context of genetic association studies that specifically aim to identify and characterize gene-gene interactions. Among these strategies is the Model-Based Multifactor Dimensionality Reduction (MB-MDR) which was first introduced by Calle et al. [1]. The strategy of MB-MDR to tackle the dimensionality problem in interaction detection involves reducing a potentially high dimensional problem to a one-dimensional problem by pooling multi-locus genotypes into three groups based on association testing or modeling (PART 1, Figure 1.4). It has been suggested that Model-Based Multifactor Dimensionality Reduction is a useful method for identifying gene-gene interactions in case-control or family-based design for both dichotomous and quantitative traits [1-6]. Although a power study of MB-MDR detection with and without main effects adjustment has been performed before by Cattaert et al. [3] and Mahachie et al. [6], these studies only involved adjusting for the known functional SNPs contributing to an epistasis effect. The preliminary results these studies gave rise to, emphasized the importance of lower-order effects adjustment when searching for gene-gene interactions and warranted a more detailed investigation, which is the topic of this chapter.

In this study, we perform a thorough simulation-based investigation of the power of quantitative trait MB-MDR to identify gene-gene interactions, using different strategies to adjust for lower-order genetic effects that may or not be part of the (functional) SNP-SNP interaction under investigation. Performance criteria used are power and family-wise error rate. We perform MB-MDR epistasis analyses first without any adjustment for main effects and then with adjustments using several strategies. The proposed main effects corrections can be grouped into two categories: 1) main effects screening followed by MB-MDR applied to an adjusted trait and 2) main effect adjustment integrated in step 1 and step 2 of MB-MDR (PART 1, Figure 1.4). These are depicted in Figure 2.1 and described in more detail in the methods section.

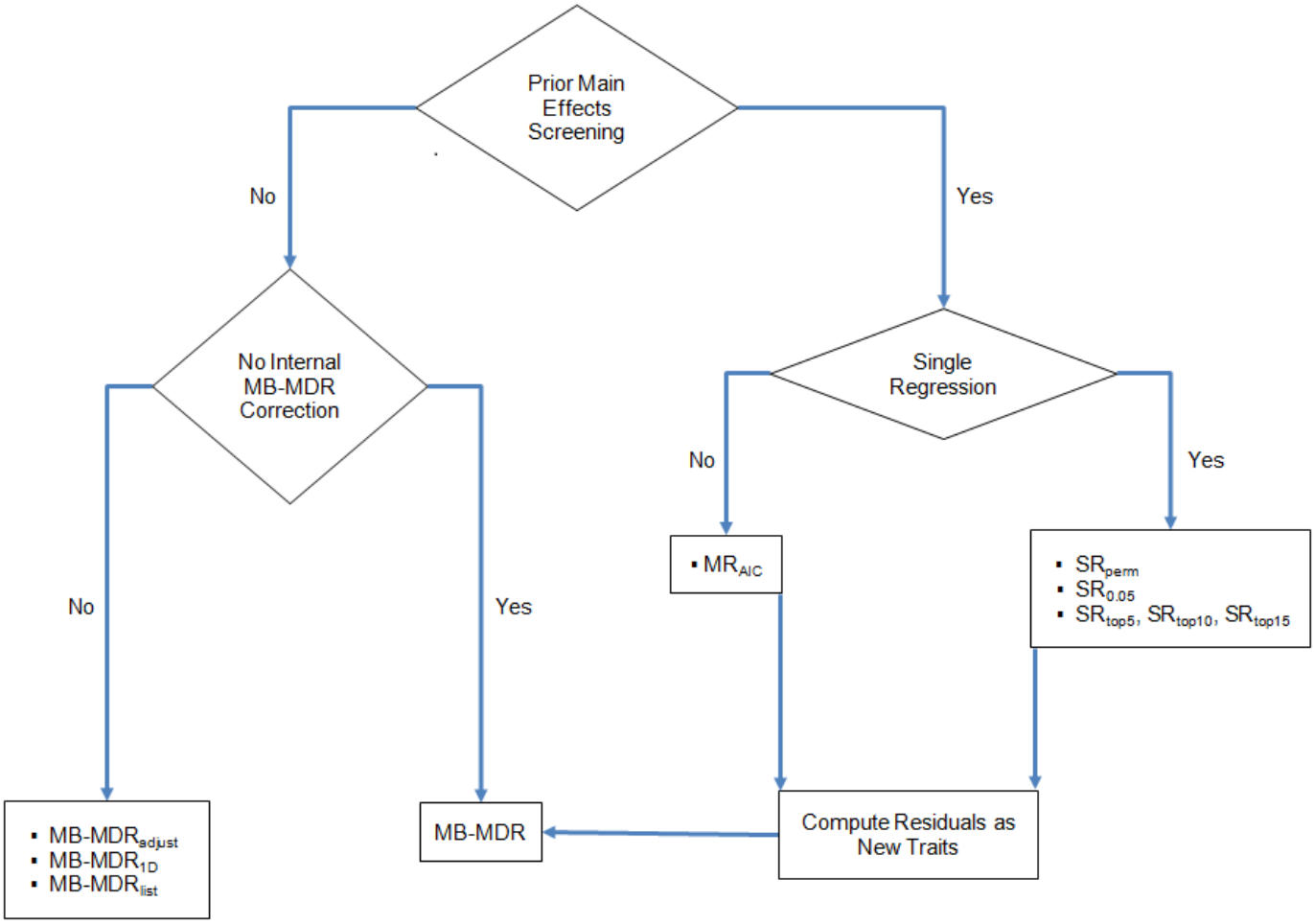

Figure 2. 1 Different approaches to adjust for lower-order effects in MB-MDR epistasis screening.

2.2 Materials and Methods

2.2.1 Strategies to adjust for lower-order genetic effects

Several methods exist to correct for lower-order effects in the context of quantitative MB-MDR epistasis screening. An overview of the considered methods in this study is given in Figure 2.1. A first strategy is to extensively look for potentially confounding main effects, to transform the original trait to an adjusted trait accordingly, and to submit this newly defined trait to MB-MDR for epistasis screening.

When correcting for main effects, a note about how to best code lower-order effects is warranted. In a GWA study, SNPs are often coded in an additive way [7]. This coding works well in practice, although power can be gained by acknowledging the true underlying genetic models [8]. For instance, if the two homozygote genotypes at a locus exhibit the same risk, different from the heterozygote risk (over-dominance), then the additive coding will have reduced power irrespective of the sample size [9]. Alternatively, several coding schemes may be investigated and a maximum statistic over screened main effects models may be selected [10]. The differing unknown operating modes of inheritance throughout the genome make it hard to flexibly and automatically acknowledge this complex inheritance spectrum.

Therefore, the route chosen in this paper, now in an epistasis context is to correct for main effects by either assuming an additive or a codominant coding scheme, in scenarios that involve different contributions of additive and dominance variance to main effects variance. Although some of these scenarios may be better captured by non-additive and non-codominant codings, the interest is in finding an all-purpose acceptable (in terms of power and type I error) way to remove the main effects signals influencing epistasis signals. Choosing between additive and codominant coding schemes implies choosing between the least and most severe such removal of effects.

2.2.1.1 Main effects screening prior to MB-MDR

This screening procedure involves first adjusting for a chosen subset of main effects via parametric (linear) regression models and then considering residuals from the fitted models as a new trait for MB-MDR. For the adjustment methods involving significance assessments, we remark that whenever none of the SNPs are significant, the original trait is submitted to MB-MDR.

Single (univariate) regression-based searches

Important main effects can be identified via single-SNP regression models, as is done in a classical GWA setting. Hence, SNPs that meet a stringent criterion (such as governed by a Bonferroni criterion) will be labelled as "important" and are therefore good candidates to correct for in an epistasis screening. In this study, we prefer to take a less conservative route, such as a selection based on step-down maxT adjusted *p*-values with 999 replicates (Figure 2.1; SR_{perm}). However, targeting effects standing out in a GWA main effects screening while maintaining overall type I error is quite different from targeting main effects to adjust for in an epistasis screening. Therefore, we also consider selecting "optimal" SNPs for main effects correction in the quantitative MB-MDR screening on the basis of their significance without correction for multiple testing (Figure 2.1; $SR_{0.05}$) or on the basis of a ranking of the corresponding raw *p*-values (Figure 2.1; SR_{top5}, SR_{top10}, SR_{top15}).

Multiple regression-based searches

Due to a large number of SNPs that are involved in a main effects genome-wide analysis, multiple regression-based searches are often automated. One such automated approach uses stepwise selection based on AIC (stepAIC in R package MASS, R 2.10.0). This procedure iteratively adds and/or drops variables to seek the lowest AIC score. The final model generates the list of main effects to correct for in the quantitative trait MB-MDR analysis (Figure 2.1; MR_{AIC}).

2.2.1.2 Main effects adjustment as an integral part of MB-MDR

In this scenario, main effects are adjusted for "on-the-fly", i.e. SNPs are adjusted for during the first 2 MB-MDR epistasis screening steps. Three types of adjustment are considered. A first type is to always adjust for the SNPs in the pair under investigation (Figure 2.1; MB-MDR_{adjust}). Hence, the adjustment is done irrespective of whether a main effect is truly present. A second type is to only adjust for SNPs that are identified by MB-MDR_{1D} as significant. Here, MB-MDR_{1D} is run first and a list of genome-wide significant SNPs is identified (based on step-down maxT with 999 permutation replicates). MB-MDR epistasis screening is then performed while only adjusting for the identified SNPs for the pair under investigation (Figure 2.1; MB-MDR_{1D}). A third type is to only adjust for significant SNPs obtained via single regression models and maxT significance assessment (Figure 2.1; MB-

MDR_{list}). Thus, for MB-MDR_{1D} and MB-MDR_{list}, any of the following 3 situations can arise: a) None of the 2 SNPs is significant and no correction is performed b) One of the 2 SNPs is significant and this is adjusted for c) Both SNPS are significant and both SNPs are adjusted for.

In order to account for potentially important SNPs as an integral part of MB-MDR, we remark that the Student's *t*-test in MB-MDR steps 1-2 (PART 1, Figure 1.4) is replaced by the Wald test for the interaction effect in a regression framework.

2.2.2 Data Simulation

Simulated data as generated in Mahachie John et al. [6] are based on two epistasis models for SNP1 and SNP2 that incorporate varying degrees of epistasis: Model M27 and Model M170 of Evans et al. [11]. In order to increase the phenotypic mean, M27 requires an individual to have at least one copy of the minor allele at both loci whereas M170 requires an individual to be heterozygous at one locus and homozygous at the other. The phenotypic means for the aforementioned epistasis models only take two values, μ_L (Low phenotypic mean) and μ_H (High phenotypic mean). The total phenotypic variance σ^2_{tot}, i.e. the sum of genetic variance at both loci $2\sigma^2_1 = \sigma^2_{main}$ (the minor allele frequencies for the functional SNPs are taken to be the same), epistasis variance σ^2_{epi}, and environmental variance σ^2_{env}, is fixed at 1. SNP1 and SNP2 have minor allele frequency equal to *p*, with *p* one of $\{0.1, 0.25, 0.5\}$. The minor allele frequencies of the other 98 markers are generated from a random uniform distribution, U(0.05,0.5). MB-MDR screening is performed on 100 SNPs in Hardy-Weinberg Equilibrium and linkage equilibrium. The total genetic variance σ^2_g is varied as $\sigma^2_g \in \{0.01, 0.02, 0.03, 0.05, 0.1\}$. The main effects variance σ^2_{main} consists of additive variance σ^2_{add} and dominance variance σ^2_{dom}. As *p* increases, the contribution to the total genetic variance of epistasis variance relative to main effects variance increases for M170 and decreases for M27, and also the contributions of additive and dominance variance to the total main effects variance change with *p* (Table 2.1).

Table 2. 1 Theoretically derived proportions of the genetic variance due to main effects (additive and dominance) or epistasis.

Model	*p*	$\sigma^2_{main}/\sigma^2_{gen}$	$\sigma^2_{add}/\sigma^2_{main}$	$\sigma^2_{dom}/\sigma^2_{main}$	$\sigma^2_{epi}/\sigma^2_{gen}$
M27	0.1	0.319	0.947	0.053	0.681
	0.25	0.609	0.857	0.143	0.391
	0.5	0.857	0.667	0.333	0.143
M170	0.1	0.581	0.780	0.220	0.419
	0.25	0.118	0.400	0.600	0.882
	0.5	0.000	0.947	0.053	1.000

For SNP3 and SNP4, main effects are imposed with associated variances σ_3^2 and σ_4^2, selected from a uniform distribution U(0, 0.06) such that the total main effects variance of the 4 loci (SNP1, SNP2, SNP3, SNP4) is $\sigma^2_{main} = 2\sigma_1^2 + \sigma_3^2 + \sigma_4^2$. The respective modes of inheritance for SNP3 and SNP4 are additive and advantageous heterozygous. Note that SNP4 will therefore contribute to both the additive and dominance components of the main effects variance. This scenario allows us to investigate the effect of global main effects correction approaches for functional SNPs that are not part of a two-locus interaction.

In addition data are simulated under the null model for the functional pair (i.e. $\sigma_g^2 = 0$) in two ways, giving rise to two null hypotheses H_{01} and H_{02}. $H_{01:}$ No genetic contribution apart from SNP3 and SNP4 as main effects and H_{02}: any genetic contribution from any of the SNPs whatsoever.

In summary, a total of 36 simulation settings are considered. For each parameter setting, we consider 500 simulation replicates, involving 2000 unrelated individuals.

2.3 Results

2.3.1 Familywise error rates and false positive rates

Table 2.2 shows results for settings simulated under the null hypotheses H_{01} and H_{02} of no genetic associations with the trait, yet in the presence or absence of additional main effects (SNP3 and SNP4).

We observe that MB-MDR type I error percentages are close to the nominal type I error rate of 5%, when no correction for main effects is performed under settings where no additional main effects act on the quantitative trait. Type I error rates are also kept under control when correction for main effects is integrated in MB-MDR epistasis screening as well as prior to MB-MDR for permutation based regression-based approach (MB-MDR$_{adjust}$, MD-MDR$_{1D}$, MB-MDR$_{list}$ and SR$_{perm}$, respectively). In particular, additive correction under H_{02} and codominant correction for both H_{01} and H_{02}. When additional main effects are present in the data, adjusting for their effects using additive correction give rise to inflated type I error rates ranging from 55 to 74%. In contrast, when adopting a codominant correction, type I error is under control for MR$_{AIC}$, and single regression-based correction methods (except SR$_{perm}$) which are extremely conservative (Table 2.2: type I error rates are close to zero).

Table 2. 2 Type I error percentages for data generated under the null hypothesis of no genetic association of the interacting pair.

Without correction and additional main effects					
P		**present**		**absent**	
0.1	**No correction**	0.982		**0.046**	
0.25		0.984		**0.050**	
0.5		0.982		**0.050**	
With Correction and additional main effects					
	Way of Correction	**Additive**		**Codominant**	
		present	**absent**	**present**	**absent**
0.1	$MB\text{-}MDR_{adjust}$	0.676	**0.048**	**0.040**	**0.052**
0.25		0.710	**0.034**	**0.054**	**0.038**
0.5		0.740	**0.044**	**0.036**	**0.050**
0.1	$MB\text{-}MDR_{1D}$	0.676	**0.036**	**0.058**	**0.030**
0.25		0.722	**0.040**	**0.042**	**0.036**
0.5		0.746	**0.040**	**0.038**	**0.030**
0.1	$MB\text{-}MDR_{list}$	0.682	**0.038**	**0.056**	**0.030**
0.25		0.726	**0.036**	**0.044**	**0.032**
0.5		0.748	**0.046**	**0.040**	**0.032**
0.1	SR_{perm}	0.628	**0.038**	**0.048**	**0.030**
0.25		0.660	**0.036**	**0.058**	**0.030**
0.5		0.678	**0.046**	**0.044**	**0.032**
0.1	$SR_{0.05}$	0.576	0.014	0.006	0.010
0.25		0.604	0.006	0.012	0.000
0.5		0.636	0.022	0.008	0.008
0.1	MR_{AIC}	0.552	0.008	0.000	0.002
0.25		0.578	0.004	0.002	0.000
0.5		0.616	0.012	0.000	0.006
0.1	SR_{top5}	0.582	0.014	0.008	0.008
0.25		0.616	0.002	0.020	0.000
0.5		0.638	0.026	0.010	0.010
0.1	SR_{top10}	0.560	0.012	0.000	0.008
0.25		0.592	0.006	0.004	0.000
0.5		0.626	0.022	0.006	0.006
0.1	SR_{top15}	0.556	0.010	0.000	0.006
0.25		0.588	0.006	0.002	0.000
0.5		0.618	0.016	0.004	0.006

Results are for scenarios: with and without additional main effects (SNP3 and SNP4) contributing to the genetic variance. In **bold** are values within Bradley's liberal criterion of robustness.

False positive rate estimates generated by MB-MDR (i.e. referring to scenarios for which one or more significantly interacting pairs other than the causal SNP pair (SNP1, SNP2)) using no correction or an additive or codominant correction of main effects, are shown in Figure 2.2. When no correction is performed, false positive rate estimates are around 100% under both M170 and M27 genetic epistasis models. In general, for additive correction false positive rate estimates range from 53 to 100% whereas for codominant correction, false positive rate estimates are lower and range from 0 to 19%. In particular, false positive rates for MB-MDR_{adjust} (always adjusting for main effects SNPs) in a codominant way range from 4 to 7%, rates that are within the interval (0.025, 0.075), satisfying Bradley's [12] liberal criterion of robustness. This criterion requires that the type I error rates are controlled for any level α of significance, if the empirical type I error rate $\hat{\alpha}$ is contained in the interval $0.5\alpha \le \hat{\alpha} \le 1.5\alpha$. For MB-$MDR_{1D}$ and MB-MDR_{list}, false positive rates are not kept under control. The actual numerical results of the false positive profiles plotted in Figure 2.2 are presented in the Appendix Table A8 for M170 and Table A9 for M27.

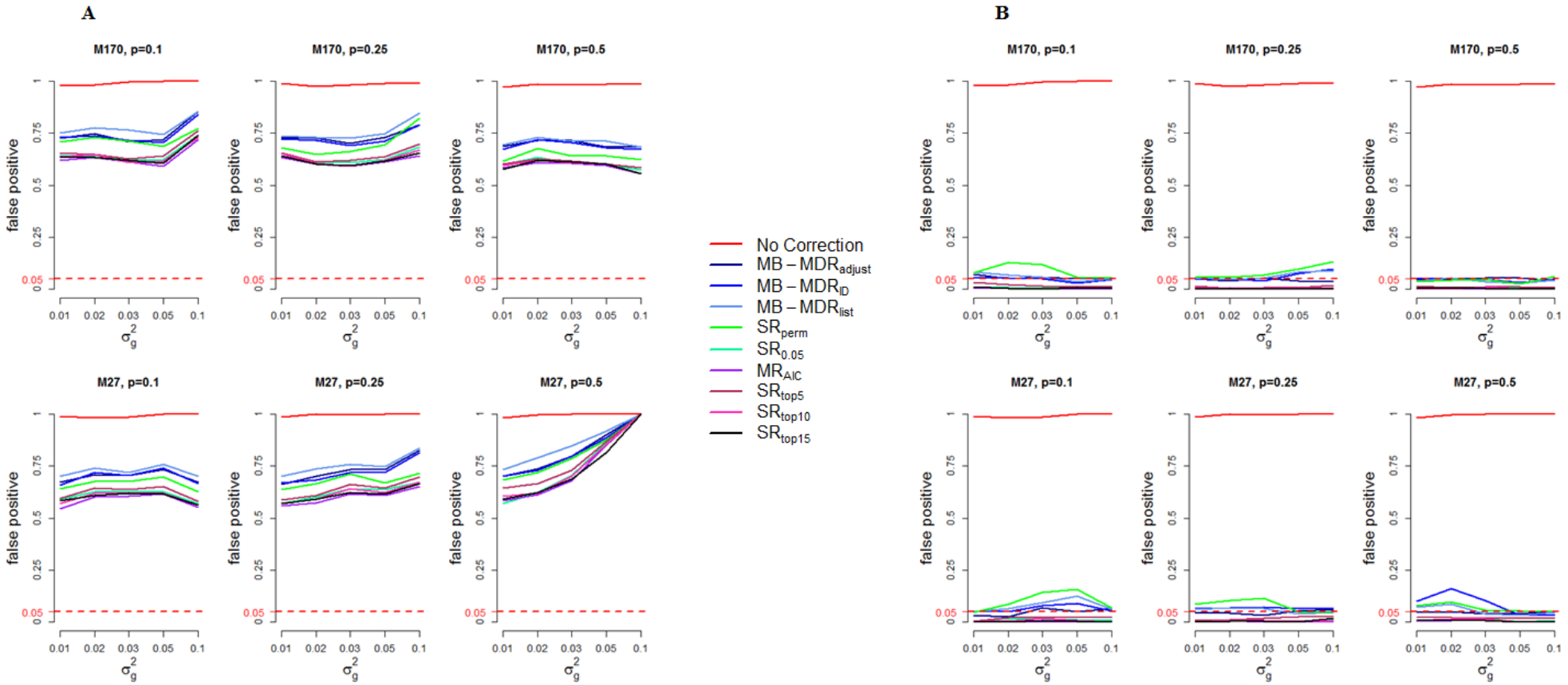

Figure 2. 2 False positive percentages of MB-MDR based on additive (A) and co-dominant (B) correction.

Legend False positive percentage is defined as the proportion of simulation samples for which pairs other than the causal pair (SNP1, SNP2) are significant

The main reason why we observe higher false positive rates under additive correction is due to the fact that SNP4 contributes to both an additive and dominance component of the main effects variance. Hence, there is also a higher chance of identifying 'significant' interactions for pairs involving SNP4. False positive rates are reduced when co-dominant correction is performed. Table 2.3 shows observed false positive rates that involve pairing with SNP3 and/or SNP4 under additive and co-dominant correction. Only MB-MDR$_{adjust}$ ("on-the-fly" adjustment) results are shown. From Table 2.3, $\sigma_g^2 > 0$, we observe that under additive codings, false positive rates range from 51 to 61% for interactions between SNP3 and SNP4. However, for interactions with SNP3 (excluding SNP3, SNP4 interaction), false positive rates range from 0 to 6%, except for Model 27, p=0.5 and σ_g^2 of 0.05 and 0.1 where false positive rates are 27 and 92%, respectively. As observed in Table 2.1, model M27, p=0.5 has the highest relative contribution of dominance variance, hence, additive correction does not fully account for SNP1 and SNP2.

Table 2. 3 False positive percentages of MB-MDRadjust involving SNP3 and/or SNP4.

	p	σ_g^2	Additive			Codominant		
			SNP3_ anyother thanSNP4	**SNP3_SNP4**	**SNP4_ anyother thanSNP3**	**SNP3_ anyother thanSNP4**	**SNP3_SNP4**	**SNP4_ anyother thanSNP3**
H_{01}	0.1	0	0.002	0.520	0.660	0.000	0.000	0.000
	0.25		0.000	0.556	0.688	0.000	0.000	0.000
	0.5		0.002	0.608	0.722	0.004	0.000	0.002
M170	0.1	0.01	0.002	0.584	0.704	0.004	0.000	0.000
		0.02	0.008	0.582	0.724	0.002	0.000	0.000
		0.03	0.000	0.572	0.690	0.000	0.000	0.000
		0.05	0.008	0.534	0.676	0.002	0.000	0.000
		0.1	0.072	0.540	0.752	0.000	0.000	0.000
	0.25	0.01	0.002	0.598	0.714	0.000	0.000	0.004
		0.02	0.000	0.558	0.712	0.002	0.000	0.002
		0.03	0.000	0.544	0.686	0.000	0.000	0.000
		0.05	0.004	0.536	0.706	0.002	0.000	0.000
		0.1	0.032	0.566	0.738	0.000	0.000	0.000
	0.5	0.01	0.000	0.526	0.664	0.000	0.000	0.002
		0.02	0.000	0.588	0.708	0.000	0.000	0.002
		0.03	0.002	0.544	0.692	0.002	0.000	0.002
		0.05	0.002	0.550	0.666	0.000	0.000	0.000
		0.1	0.002	0.528	0.662	0.002	0.000	0.000
M27	0.1	0.01	0.002	0.532	0.662	0.000	0.000	0.000
		0.02	0.000	0.564	0.690	0.000	0.000	0.000
		0.03	0.000	0.554	0.680	0.002	0.000	0.000
		0.05	0.000	0.562	0.704	0.002	0.000	0.000
		0.1	0.000	0.518	0.638	0.000	0.000	0.000
	0.25	0.01	0.002	0.512	0.652	0.000	0.000	0.002
		0.02	0.004	0.520	0.682	0.004	0.000	0.000
		0.03	0.000	0.562	0.700	0.002	0.000	0.000
		0.05	0.000	0.546	0.700	0.000	0.000	0.002
		0.1	0.042	0.564	0.734	0.002	0.000	0.000
	0.5	0.01	0.000	0.546	0.672	0.000	0.000	0.002
		0.02	0.020	0.508	0.684	0.000	0.000	0.000
		0.03	0.060	0.518	0.706	0.000	0.000	0.002
		0.05	0.272	0.536	0.806	0.000	0.000	0.000
		0.1	0.912	0.590	0.974	0.000	0.000	0.000

False positive percentages shown are for identifying interaction between SNP3 and SNP4 and or interactions between SNP3 or SNP4 and at least one other SNP for null data scenario under H_{01} and for models M170 and M27.

2.3.2 Empirical power estimates

Power profiles of MB-MDR to detect the correct interacting pair (SNP1, SNP2) without and with different ways of adjustment of main effects are shown in Figure 2.3. Empirical power estimates are presented as Supplementary information (Table A8 for M170 and Table A9 for M27). In this section, we focus on scenarios where there is some remarkable degree of main effects contributing to the genetic variance (M170: p=0.1, M27: p=0.25 and 0.5). For a detailed view on variance decomposition into main and epistatic effects, we refer to Sham [13]. Under the aforementioned scenarios, the profile for no correction always has the highest power.

Under M170, the empirical power estimates for this profile range from 33 to 100% for p=0.1. Under M27, the power estimates range from 27 to 100% and from 15 to 100%, for p=0.25 and 0.5 respectively. Irrespective of whether main effects are corrected for using additive or codominant coding, profiles for the considered multiple-regression, MR_{AIC} and single regression-based methods that do not involve multiple testing ($SR_{0.05}$, SR_{top5}, SR_{top10} and SR_{top15}) tend to follow the same trajectory, giving rise to the lowest empirical power estimates. With additive adjustments, empirical power estimates for these corrective ways range from 0 to 100% for both models M27 and M170. With codominant adjustments, power estimates range from 0 to 94% , for M170, p=0.1, from 0 to 100% and from 0 to 18%, for model M27, p=0.25 and p=0.5 respectively. Estimates for MB-MDR_{adjust} (corrective methods that are integrated as part of MB-MDR) , range from 6 to 100% for M170, p=0.1, from 3 to 100% for M27 with p=0.25 and from 1 to 100% for M27 with p=0.5, when additive corrections are performed. Under codominant corrections the estimates range from 4 to 100% for M170 (p=0.1) and from 4 to 100% or from 0 to 68% for M27 (p=0.25 and p=0.5 respectively).

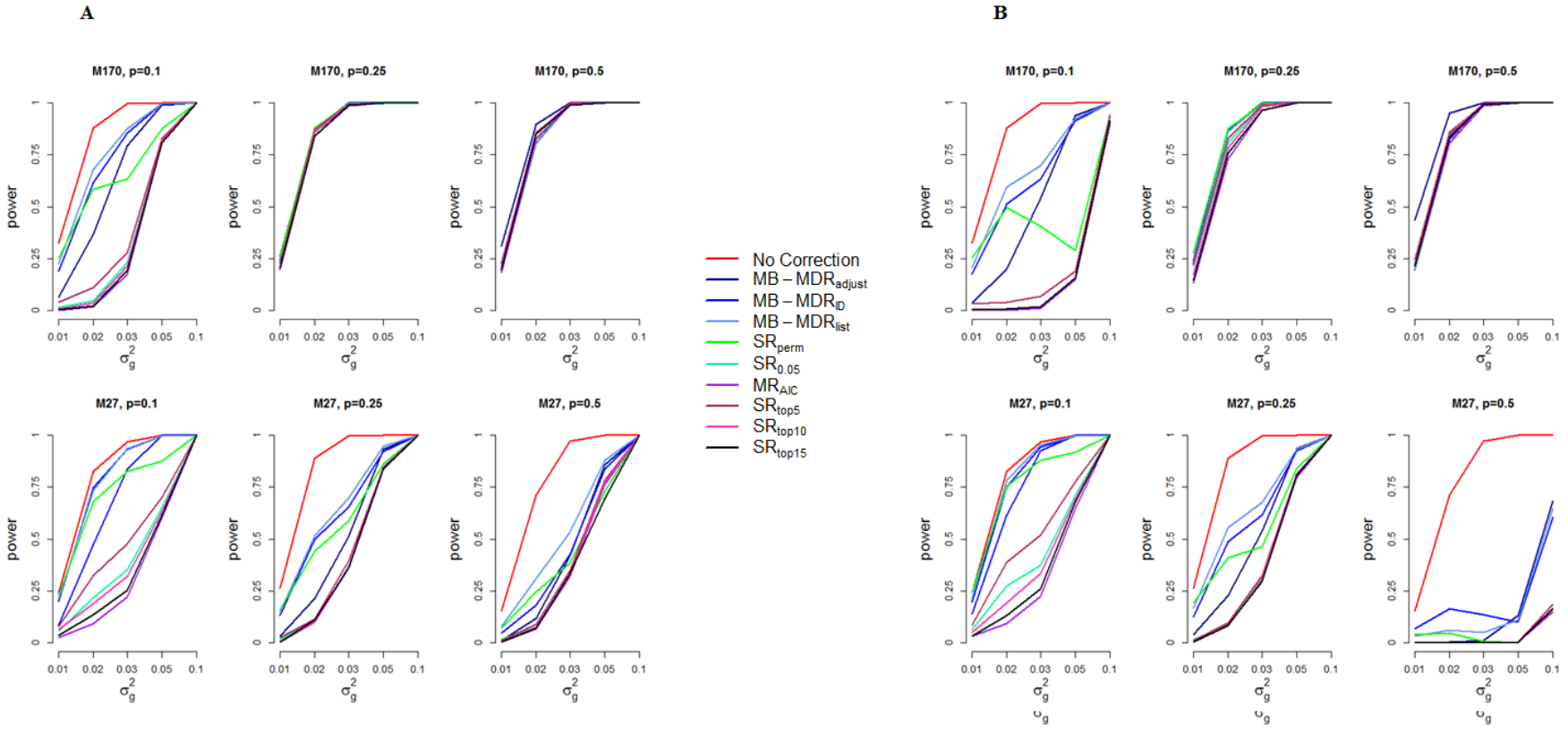

Figure 2. 3 Power to identify SNP1, SNP2, as significant for additive (A) and codominant (B) correction.

2.4 Discussion

The identification of genetic susceptibility loci for human complex diseases has been rather successful due to the ability to combine different genome-wide association studies via meta-analyses. In the quest for the missing heritability, genome-wide association interaction studies have become increasingly popular and the field shows a boost in methodological developments [14]. When lower-order effects are not appropriately accounted for in epistasis screening, derived results may not be trustworthy and conclusions about genuine epistasis may be ungrounded.

Indeed, the challenge is to find epistasis effects above and beyond singular marker contributing effects, should there be any. In this work, we investigated the power of MB-MDR for quantitative traits and unrelated individuals, while targeting gene–gene interactions accounting for potential main effects.

As was already observed in Mahachie John et al. [6], MB-MDR adequately controls type I rate at 5% when no association is present (null data). Under additive corrections, type I error and false positive rates are high irrespective of the adjustment method considered but controlled under codominant corrections. This is due to the existence of SNP4, which was simulated with both additive and dominance effects (advantageous heterozygous). Hence, additive adjustment does not fully remove the effect of SNP4. As shown before in Table 2.3, the consequence is that a number of SNPs appear to be significantly interacting with SNP4. Not surprisingly, this occurs more often under additive correction compared to codominant correction. This is because when we correct for main effects using the codominant model, we remove all the effect of SNP4, and hence false positive results are only by chance (5% nominal error rate). When no main effects adjustment is implemented, MB-MDR gives even higher false positive rate rates.

Lower power profiles under codominant corrections in Figure 2.3 are explained by the different contributions of additive and dominance effects to the total main effects variance as already shown in Table 2.1. When there is a remarkable contribution of dominance effect, as mentioned before, additive coding does not fully remove main effect contribution of the interacting SNPs. For instance, under M27, when the contribution of main effects is maximum (p=0.5), almost 33% of the main effects variance is dominance, hence a huge difference in the power profiles between additive and codominant codings.

Interestingly, easy-to-use automatic subset selection procedures (MR_{AIC}) and single regression-based identification of important main effects prior to MB-MDR screening result in lower power and almost zero false positive rates. Often, a list of top SNPs is generated to derive disease genetic risk scores. Some of these SNPs may reach user-defined significance, some may even reach genome-wide significance and some may not be significant at all. Hence, correcting for SNPs in such a list (e.g. top5, 10, 15) may remove more of the trait's variability than is really necessary, especially when correction for multiple testing is not performed. Note that we considered a minimum of 5 top findings since at least 4 SNPs were allowed to contribute to the main effects variance.

In order to attain sufficient power, any main effects corrective method that leads to an over-correction during epistasis screening should be avoided. All considered residual-based approaches (MR_{AIC}, $SR_{0.05}$, SR_{perm}, SR_{top5}, SR_{top10}, SR_{top15}) led to uncontrolled false positive rates. This can be explained by either the way the residuals were obtained (inappropriate main effects coding) or by the non-exhaustive list of markers considered in the residual computation.

Only codominantly correcting for significant SNPs as integral part of MB-MDR screening perform much better. However, the poor performance of MB-MDR_{1D} and MB-MDR_{list} and the excellent performance of MB-MDR_{adjust} in terms of controlling false positive epistasis rates support the intuition that it (only) matters to correct for those SNPs that are involved in the SNP pair under investigation, when no other SNPs are expected to modify the effect of that pair.

The aforementioned discussion clearly raises questions about how to best correct for lower-order effects when higher-order (>2) interactions are targeted. In either case, to aid in interpretation of results, it is always a good practice to assess the joint information of clusters of SNPs that contribute to the trait variability [15].

Finally, we emphasize that most statistical epistasis detection methods can be decomposed into a core component and a multiple testing correction component. Keeping the core component, but using a more refined multiple testing correction can generally enhance its performance. For instance, assumptions underlying the maxT procedure of Westfall and Young [16] that is implemented in MB-MDR are likely to be violated for MB-MDR_{1D} and MB-MDR_{list}. Indeed, the null and the alternative hypotheses per pair of SNPs under investigation are no longer the same for all interaction tests.

In conclusion, rather than adjusting for lower-order effects prior to MB-MDR and using residuals as the new trait, or adjusting only for significant SNP(s), we advocate an "on-the-fly" main effects adjustment (MB-MDR$_{adjust}$). This type of adjustment only removes potential main effects contributions in the pair under investigation but keeps the null and alternative hypotheses similar from one pair of SNPs to another. We have shown that the commonly used additive coding in the "on-the-fly" adjustment (MB-MDR$_{adjust}$) is not sufficient and leads to overly optimistic results and that codominant adjustments are to be preferred. This will ensure an acceptable balance between type I error and power to identify the interactions.

Realistic settings often involve both additive and dominance genetic effects to the trait under investigation. Equivalent to our codominant coding, a perhaps biologically more meaningful coding involves introducing 2 variables X_1 and X_2 with values -1, 0, 1 and -1/2, 1/2, -1/2, respectively, for homogenous wild type, heterozygote and homozygote mutant genotypes. In such a coding scheme, both additive and dominant scales are represented. This 2-parameter coding is statistically attractive since it is invariant to allele coding (i.e. whether coding homogenous wild type as 1 or homozygote mutant genotypes as 1 for X_1) [17]. The utility of the aforementioned coding as a way to adjust for lower-order effects in MB-MDR higher-order epistasis screening will be the subject of future research.

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Chapter 3

A Robustness Study of Parametric and Non-parametric Tests in Model-Based Multifactor Dimensionality Reduction for Epistasis Detection

Related publication
Jestinah M. Mahachie John, François Van Lishout,
Elena S. Gusareva, Kristel Van Steen (2012)
Under Revision

Abstract

Applying a statistical method implies identifying underlying (model) assumptions and checking their validity in the particular context. One of these contexts is association modeling for epistasis detection. Here, depending on the technique used, violation of model assumptions may result in increased type I error, power loss, or biased parameter estimates. Remedial measures for violated underlying conditions or assumptions include data transformation or selecting a more relaxed modeling or testing strategy. Model-Based Multifactor Dimensionality Reduction (MB-MDR) for epistasis detection relies on association testing between a trait and a factor consisting of multilocus genotype information. For quantitative traits, the framework is essentially ANalysis Of VAriance (ANOVA) that decomposes the variability in the trait amongst the different factors. In this study, we assess through simulations, the cumulative effect of deviations from normality and homoscedasticity on the overall performance of quantitative Model-Based Multifactor Dimensionality Reduction (MB-MDR) to detect 2-locus epistasis signals in the absence of main effects. Our simulation study focuses on pure epistasis models with varying degrees of epistatic influence on a quantitative trait. Conditional on a multilocus genotype, we consider quantitative trait distributions that are normal, chi-square or Student's *t* with constant or non-constant phenotypic variances. All data are analyzed with MB-MDR using the built-in Student's *t*-test for association, as well as a novel MB-MDR implementation based on Welch's *t*-test. Traits are either left untransformed or are transformed into new traits via logarithmic, standardization or rank-based transformations, prior to MB-MDR modeling. The simulation results show that MB-MDR controls type I error and false positive rates irrespective of the association test considered. Empirically-based MB-MDR power estimates for MB-MDR with Welch's *t*-tests are generally lower than those for MB-MDR with Student's *t*-tests. Trait transformations involving ranks tend to lead to increased power compared to the other considered data transformations. When performing MB-MDR screening for gene-gene interactions with quantitative traits, we recommend to first rank-transform traits to normality and then to apply MB-MDR modeling with Student's *t*-tests as internal tests for association.

3.1 Introduction

The search for epistasis or gene-gene interaction effects on traits of interest is marked by an exponential growth. From an application point of view, these searches often focus on candidate genes or pathways. The reasons for this are often logistic ones: First, genome-wide screening for epistasis requires large sample sizes to ensure sufficient power detection, which may only become available when having access to consortia data. Second, exhaustive genome-wide epistasis screenings assumes the availability of sufficient computer power and an adequate infrastructure to store and analyze the data, as well as to store and process the analysis results. From a methodological point of view, searches for epistasis effects are performed with the goal in mind to develop methods that can narrow the gap between statistical and biological epistasis.

To date, several epistasis detection approaches exist, each addressing differential aspects of the underlying theoretical epistasis model, and with different performances in terms of Type I error control or power detection [1]. Although methods are often thoroughly compared to competing methods in this sense, using a variety of simulation settings that are hoped to reflect realistic mechanisms of disease-causing genetic variants, they usually do not involve comprehensive statements neither about the underlying assumptions, nor about how violations of these assumptions may affect the method's performance. Modeling or testing techniques usually come with specific assumptions that need to be fulfilled in order to produce valid analysis results. This also applies to methods to detect epistasis. Good standard practice in this context would include 1) to investigate the underlying assumptions of the epistasis detection or modeling technique, 2) to check whether these are valid, and 3) to take remedial measures or to accommodate the effects of identified violations.

Model-Based Multifactor Dimensionality Reduction (MB-MDR) is an intrinsic non-parametric method since no assumptions are made regarding genetic modes of inheritance [2]. The 'modeling' part in MB-MDR arises from the need to embrace parametrics when adjusting for lower-order (main) effects within a regression framework. The necessity of lower-order effects corrections in quantitative MB-MDR analyses has been discussed elsewhere [3]. In pure epistasis scenarios (i.e., no significant main effects), there is no need to adjust for main effects and MB-MDR analysis essentially involves the consecutive application of one-way Analysis of Variance (ANOVA) F-tests that compare (groups of) multi-locus genotype cells with respect to the quantitative trait under study. Note that the result of a *t*-test is identical to

that of an ANOVA computed for two groups; the *t*-statistic is the square root of the F-statistic used in ANOVA. Hence, in principle, the validity of MB-MDR epistasis results may depend on whether or not ANOVA assumptions are met, which warrants further investigation. Many authors have studied the effects of model violations in regression settings in general and have suggested alternative strategies when violations cannot be remediated [4, 5].

Due to the aforementioned link between MB-MDR and ANOVA, we are particularly interested in violations regarding the latter. The three main ANOVA assumptions are: 1) the observations are independent, 2) the sample data have a normal distribution within factor levels (e.g., multilocus genotype classes) and 3) the dependent variable's variances within each factor level are homogeneous (homoscedasticity) [4]. Generally speaking, when either the assumption of normality or homoscedasticity or both are violated, highly inflated type I errors and false positives can arise, suggesting a non-robustness of parametric methods under these scenarios [6]. It should be noted though that F- and *t*-tests are scarcely affected by non-normality of population distributions (e.g, [7, 8]). Nevertheless, when the dependent variable does not meet ANOVA's normality assumption, the non-parametric Kruskal-Wallis or Mann-Whitney U test [9] is commonly taken to replace the ANOVA's F or a Student's *t*-test. However, these non-parametric counterparts are not an immediate solution to the problem of unequal variances (heteroscedasticity), as was shown before [10-12]. Alternatively, data transformations can be considered to induce normality. For instance, Wolfe et al. [13] used the logarithmic transformation to transform a skewed distribution to a distribution that is approximately normal. On the other hand, Jin et al. [14] highlighted that, when the logarithmic transformation is used, it may over-compensate right-skewed data and create left-skewed data, which can hardly be seen as an improvement. The Mann-Whitney U test avoids making distributional assumptions other than requiring group distributions of identical shape. For two-group comparisons, it is equivalent to an ordinary Student's *t*-test performed on the ranks of the original outcome measurements and its *p*-values are mathematically identical to Kruskal–Wallis one-way analysis of variance by ranks [15, 16]. The additional difficulties with data transformations prior to analysis (whether based on ranks or not) are that a chosen transformation may not address all issues at once (this is: addressing non-normality and unequal variances), and that several linear or non-linear data transformations will have different implications on post-analysis interpretability. A road map for the appropriate use of non-parametric and parametric two-group comparison tests when group sizes are equal is given in Appendix Figure A3.

The event of unbalanced data (i.e., unequal sample sizes in group comparisons) affects the choice for a particular test as well. Gibbons and Chakraborti [17] emphasized that for unbalanced ANOVA designs, Mann–Whitney U tests are not a suitable replacement for Student's *t*-tests when variances are unequal, irrespective of whether the assumption of normality is satisfied or violated. When normality and homogeneity of variance are violated together, Zimmerman and Zumbo [18] suggest that the Welch's *t*-test, alias the unequal variance *t*-test, can effectively replace the Mann–Whitney U test when the data are first transformed to ranks prior to testing. However, it has been reported in Danh [19] that the test with Welch correction becomes too conservative when sample sizes are strongly unequal compared to the Student's *t*-test. Instead, Szymczak [20] and Rupar [21] suggest focusing on medians (e.g. Mood's Median test). However, Pett [22] has argued that medians tests are less powerful than other non-parametric tests (e.g. Mann-Whitney and Kruskal-Wallis one-way ANOVA by ranks) because these only use two possibilities for scores: scores either above or below/equal to the median and the absolute value of the difference between the observed scores and the median is not accounted for. Figure 3.1 summarizes the utility of some popular parametric and non-parametric two-group comparison tests when group sizes are unequal [23].

In the context of genetics, model violations and effects of imbalanced data have primarily been discussed in the context of gene expression studies and *t*-test/ANOVA models (e.g., [20, 24, 25] . The topic is severely under-appreciated in the context of epistasis screening, as indicated before. For the latter, violations may pertain to prioritization or pre-screening algorithms, to the actual epistasis modeling and testing, as well as to the implemented corrections for multiple testing. Also for MB-MDR it has never been investigated what the *cumulative* effect is of violated association test assumptions, acknowledging that the presence and extent of these violations may differ within and between several stages of the MB-MDR analysis. However, concerns about distributional data assumptions for MB-MDR association testing can easily be removed by adopting a non-parametric viewpoint based on ranks (Figure 3.1). In this study, we use simulations to assess the cumulative effect of deviations from normality and homoscedasticity on the overall performance of quantitative Model-Based Multifactor Dimensionality Reduction (MB-MDR) with variable association tests to detect 2-locus epistasis signals. We investigate the utility of data transformations to maintain or to increase MB-MDR's efficiency and to control false positive rates. Since important lower-order genetic effects not adjusted for can also give rise to inflated type I errors or false

positive epistatic findings, as discussed in [2, 3], we restrict our attention to pure epistasis two-locus models (i.e., no main effects).

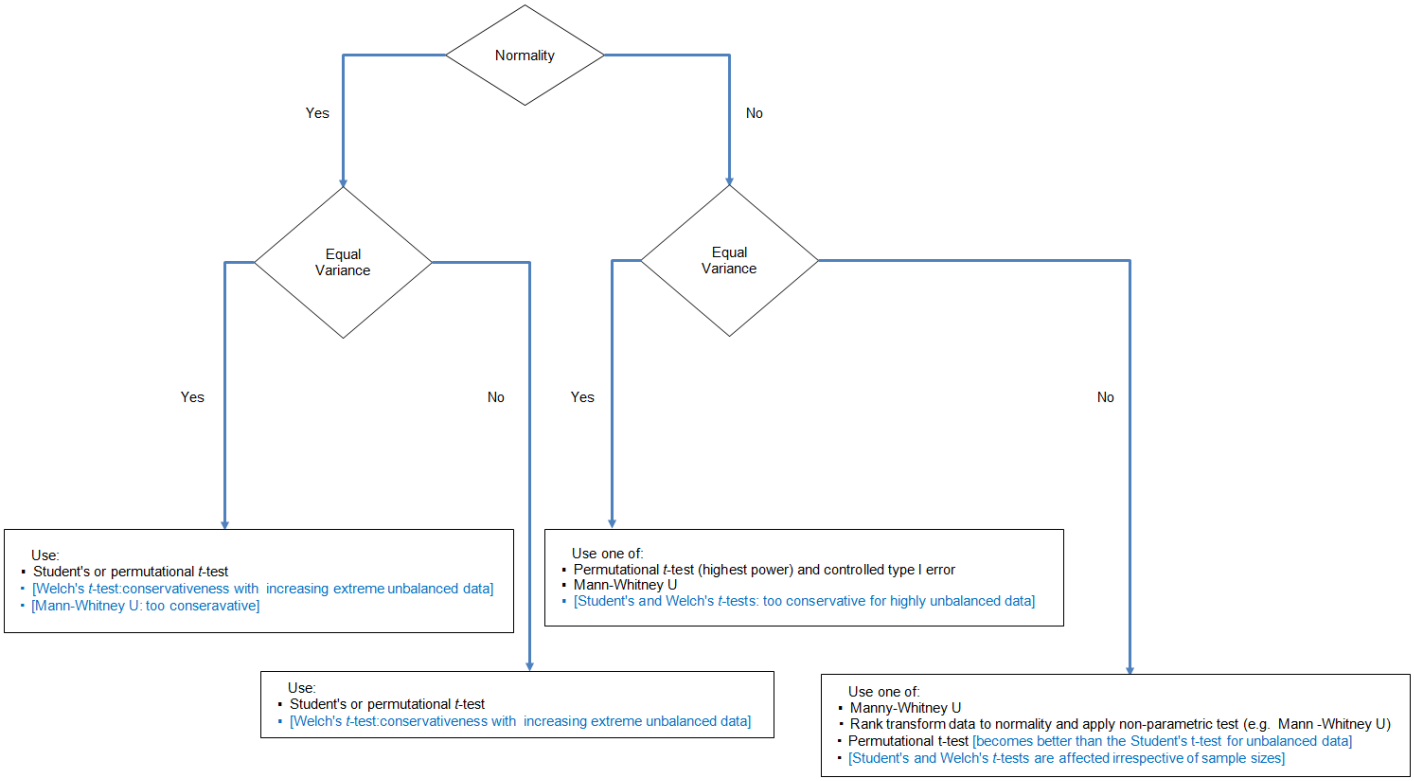

Figure 3. 1 Group comparison test, maintaining adequate Type 1 error control, when group sizes are unequal.

Legend When several tests are listed, they are listed from most (top) to least (bottom) powerful. The tests in a square box and blue font should be avoided in MB-MDR due to reasons mentioned next to them.

3.2 Materials and Methods

3.2.1 Analysis method: MB-MDR

Model-Based Multifactor Dimensionality Reduction (MB-MDR) is a data mining technique that enables the fast identification of gene-gene interactions among 1000nds of SNPs, without the need to make restrictive assumptions about the genetic modes of inheritance. The most commonly used implementation of MB-MDR involves testing one multi-locus genotype cell versus the remaining multi-locus cells (i.e. 1 cell versus 8 remaining cells, in case of 2 bi-allelic loci). By construction, this procedure creates two (possibly highly) imbalanced genetic groups that subsequently need to be compared in terms of mean or median trait differences. To date, MB-MDR has used Student's *t*-test to make such group comparisons for quantitative traits. This implementation is based on simulation studies that assumed traits to be normally distributed with equal genotypic variances for each of the multi-locus genotype combinations corresponding to a bi-allelic functional SNP pair [2, 3]. The MB-MDR outputted final test statistics for epistasis evidence are presented as ANOVA F-statistics. Naturally, different numbers of individuals contribute to specific multilocus genotype combinations. More importantly, MB-MDR's internally performed group comparison tests involve possibly highly unequal group sizes. Hence, parametric *t*-tests are always pooled variance *t*-tests. A novel implementation allowing unequal group variances based on the Welch's *t*-test (WT) for two-group comparisons is included in the MB-MDR software *version 2.7.4*.

All simulated data are analyzed with MB-MDR, with Student's *t*-test (ST) as well as the novel Welch's *t*-test (WT) implementation to assess power and type I error. Prior to MB-MDR submission, original traits are either left untransformed or transformed into new traits via logarithm transformations (Log), standardization transformation (Stz) or via rank-based transformations. The latter transformations involve the assignment of absolute ranks to all available trait measurements in a serially increasing order (Rank), after which the ranks are transformed to normality (Rtn). Data transformations are conducted in R.2.15.0 [26]. We are currently working on a MB-MDR version that will optionally use a rank-transformation of original trait values, allowing MB-MDR analyses with parametric *t*- or non-parametric Mann-Whitney U- tests of association. Overall significance is assessed at 5% level of significance after correction for multiple testing via the permutation-based step-down maxT multiple testing correction of [27] (see also [28]). Permutations are based on 999 new data replicates.

Small group sizes in group comparisons are dealt with by requiring a minimum contribution of 10 individuals to each group.

3.2.2 Data Simulation

We simulate 18 two-locus settings of an epistasis model following Evans et al. [29], each setting involving 1000 replicates and consisting of 500 unrelated individuals per replicate. In particular, simulations are based on model M170 which requires an individual to be heterozygous at one locus and homozygous at the other in order to have an increased quantitative phenotype. Minor allele frequencies (MAFs) for the causal epistatic pair (SNP1 and SNP2) are prespecified at 50%, hereby a pure epistasis model (M170 becomes a pure epistasis model when the MAFs of the two SNPs are set at 50%). An additional 98 SNPs are generated with MAFs randomly sampled from a uniform distribution; U(0.05,0.5). We assume all SNPs to be in Hardy-Weinberg Equilibrium and assume linkage equilibrium between them. The proportion of phenotypic variation that is due to epistatic variation (σ_g^2) between individuals is varied as 0, 5 and 10%.

To assess the effect of violated normal trait distributions, we consider trait distributions that are, apart from normal, also chi-squared or Student's *t*; the same distribution is assumed for each of the 9 levels of the two-locus genotypes derived from SNP1 and SNP2 combined. To investigate the MB-MDR cumulative effects of heteroscedasticity, we consider for every aforementioned setting, constant and non-constant phenotypic variances according to the following scenarios.

Scenario1: Normal distribution

We simulate 9 variances from U[1,10], one for every two-locus genotype combination corresponding to SNP1 and SNP2. Homoscedasticity or constant variance is induced by simulating traits with multi-locus specific variance equal to the average of the 9 genotypic variances mentioned before.

Scenario 2: Chi-square distribution

Quantitative traits are generated from a central chi-square distribution with 2 degrees of freedom (df), inducing a constant trait variance for every two-locus genotype combination. To simulate settings with heteroscedasticity, non-central chi-square distributions are used, df randomly selected from the uniform distribution U[2, 10]. The non-centrality parameter (ncp)

for every two-locus genotype combination is taken to be the difference between a preset maximum (maxncp) of 10 and the genotype combination-specific df. This results in a constant trait mean for all multi-locus genotypes (equal to maxncp) and phenotypic variances (twice the df + 4 times the ncp) ranging from 20 to 36.

Scenario 3: t-distribution

We consider quantitative traits from a *t*-distribution with 3 degrees of freedom. Non-equal phenotypic variances are introduced by generating data for the 9 multilocus genotype combinations from the uniform distribution U[3, 10].

3.3 Results

3.3.1 Data related

Figure 3.2 shows density plots for the normal and chi-squared distributed original data (panel A) and rank-transformed to normality traits (panel B) with equal and unequal variances. The 9 density groups refer to the 9 possible multi-locus genotypes the causal SNP pair and are based on a single replicate, so as to keep the total sample size to 500 individuals. For each scenario, the first generated dataset was used. Cell 0-0 on row 1 and column 1 (cell 2-2 on row 3 and column 3) refers to homozygous most (least) frequent allele individuals. The contribution of the epistatic variance to the trait variance is 10%. Other replicate data or assumptions about epistatic evidence give rise to similar plots (not shown). Rank-transformation to normality (Rtn) (cfr. panel B) effectively deals with multimodal data distributions (cfr. panel A). Testing whether the multilocus genotype-specific traits can be assumed to come from a normal population (Shapiro-Wilk's test) highlights a successful transformation from potentially non-normal data (panel A) to approximate normal data (panel B).

For the same scenarios as before, yet using all SNP pairs, and the 999 permutations F-statistics data, we create quantile-quantile plots (qq-plots) for a theoretical F distribution with (g-1, n-g) degrees of freedom. Here, n=500 is the number of individuals in a dataset and g=2 is the number of groups (i.e. 1 cell versus 8 remaining cells). Note that since no missing data were considered, all theoretical distributions for Student's *t* association tests within MB-MDR, whatever SNP pair is considered, should be F(1,498). Whereas Figure 3.3 shows the qq-plots for association tests (squared Student's *t*) comparing a single multi-locus genotype (in particular, cell 0-0) with the 8 remaining ones, Figure 3.4 shows the qq-plots related to the SNP pairs and their MB-MDR step 2 test statistics (i.e., the maximum of association tests involving *H*-cells versus {*L*,*O*}-cells and one comparing the *L*-cells versus {*H*,*O*}-cells). Comparison of Figure 3.3 with Figure 3.4 could suggest that deviations from a theoretical F-distribution is not so much of a concern at MB-MDR's dimensionality reduction step (i.e., labeling of multilocus genotypes according to "severity"), but seems to be quite dramatic for MB-MDR's step 2 two-locus test. This observation can be made, irrespective of whether traits initially are normally or chi-squared distributed, and irrespective of whether the original traits or rank-transforms to normality are considered.

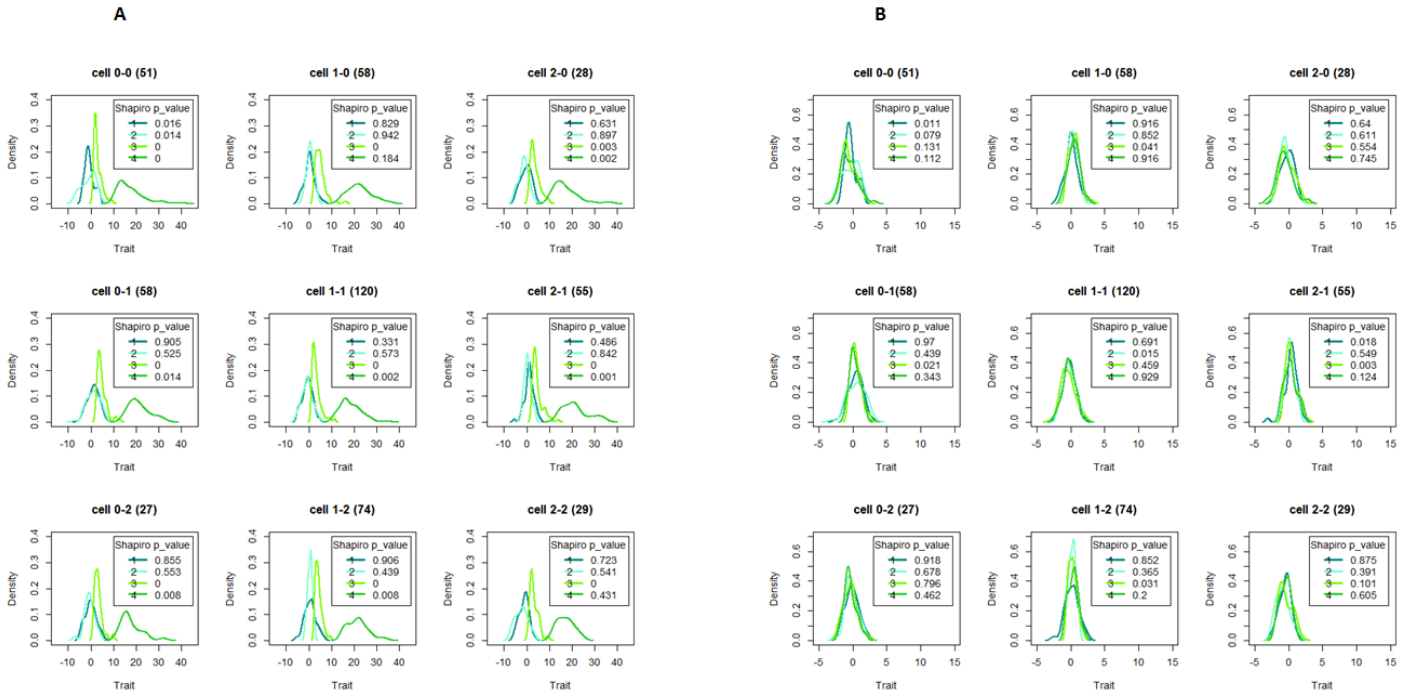

Figure 3. 2 Density plots for original trait (panel A) and rank transformed traits (panel B) for one simulated data replicate with epistatic variance, 10%.

Legend Numbers as they appear with color lines in the legend: 1=normal constant variance, 2=normal non-constant variance, 3=chi-square constant variance, 4=chi-square non-constant variance. Wild-type individuals (homozygous for the major allele) are coded as 0, heterozygous individuals as 1, and individuals homozygous for the minor allele as 2. Figures in brackets represent sample sizes for the multi-locus genotype cells.

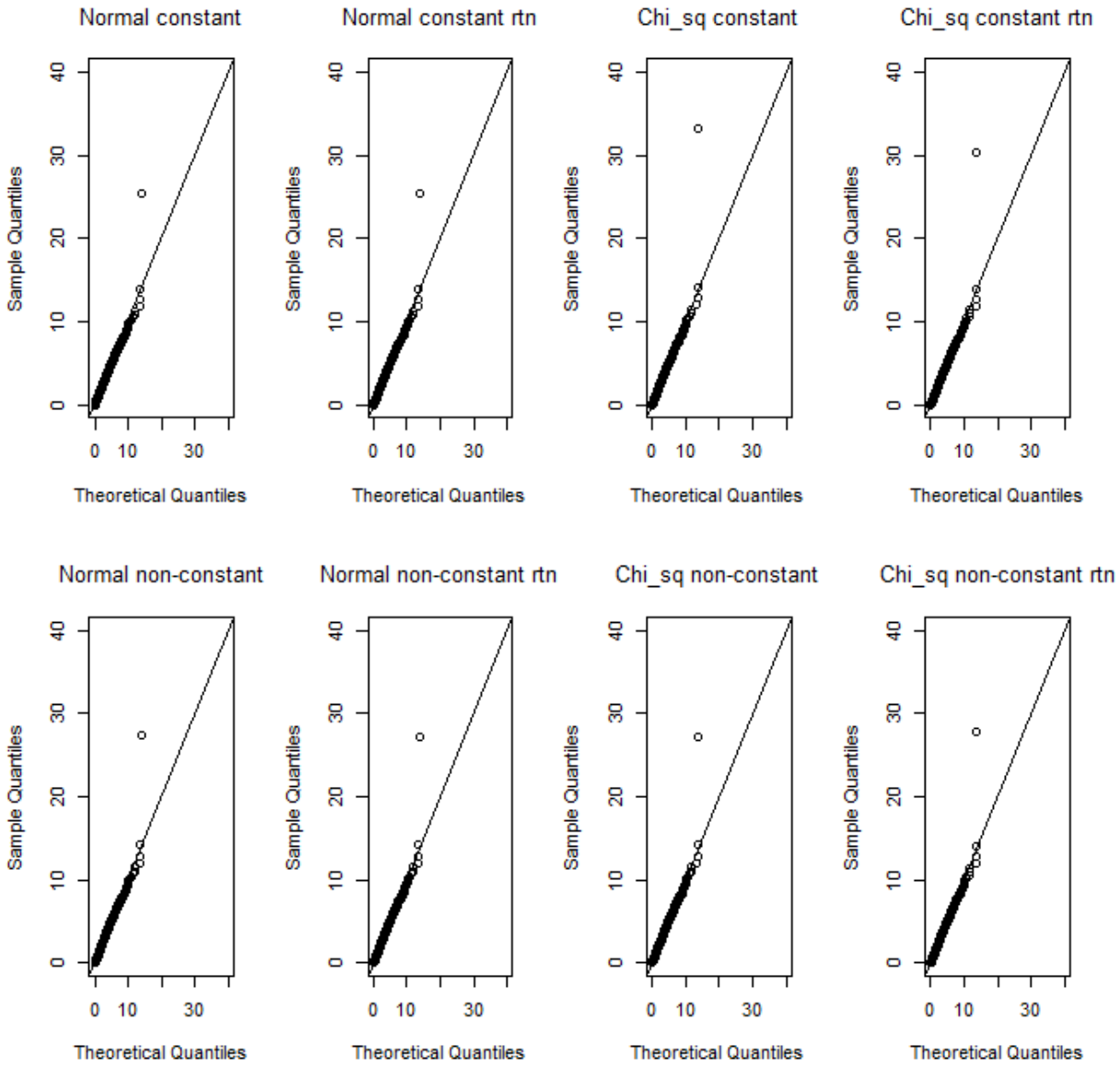

Figure 3. 3 Qq-plots of squared Student's t- test values for association between the multi-locus genotype combination cell 0-0 versus the pooled remaining multi-locus genotypes, for normal and chi-squared trait distributions or non-transformed and rank-transformed to normal data.

Legend Each time, one replicate with epistatic variance, 10% is considered and F-statistics are pooled for all SNP pairs over the 999 permutations. A theoretical F-distribution according to F(1,498) is taken as the reference.

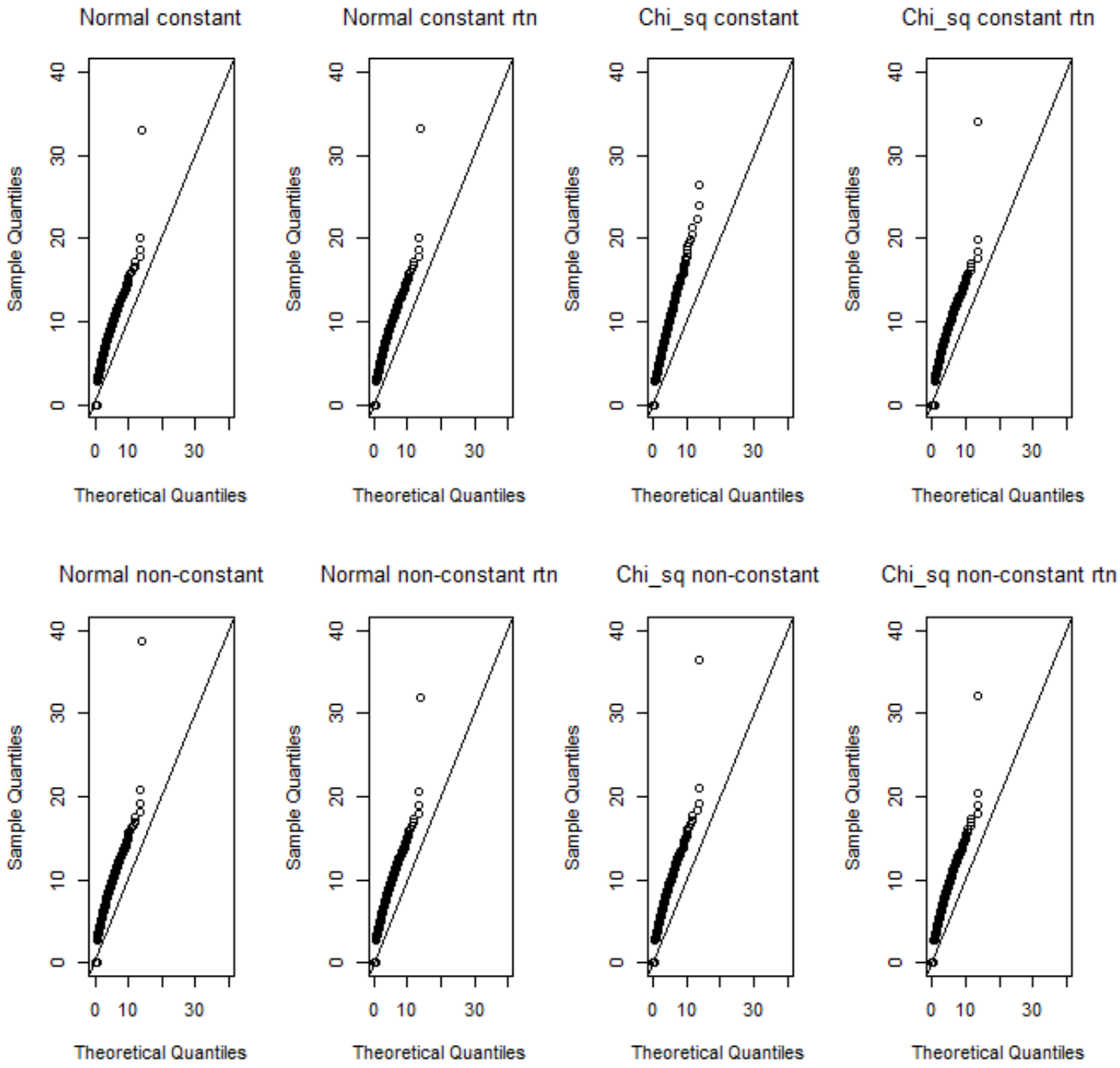

Figure 3. 4 Qq-plots of MB-MDR step 2 test values (squared Student's t), for normal and chi-squared trait distributions, and non-transformed or rank-transformed to normal data.

Legend For each setting, one replicate with genetic variance, 10% is considered and F-statistics are pooled for all SNP pairs over the 999 permutations. A theoretical F-distribution according to F (1,498) is taken as the reference.

However, recreating Figure 3.3, now for cell (2,2) instead of (0,0) (hence, the multilocus genotype cell which has the smallest number of individuals contributing to it), also highlights hard to ignore deviations from the theoretical F(1,498) distribution at cell labeling stage (see Figure 3.5).

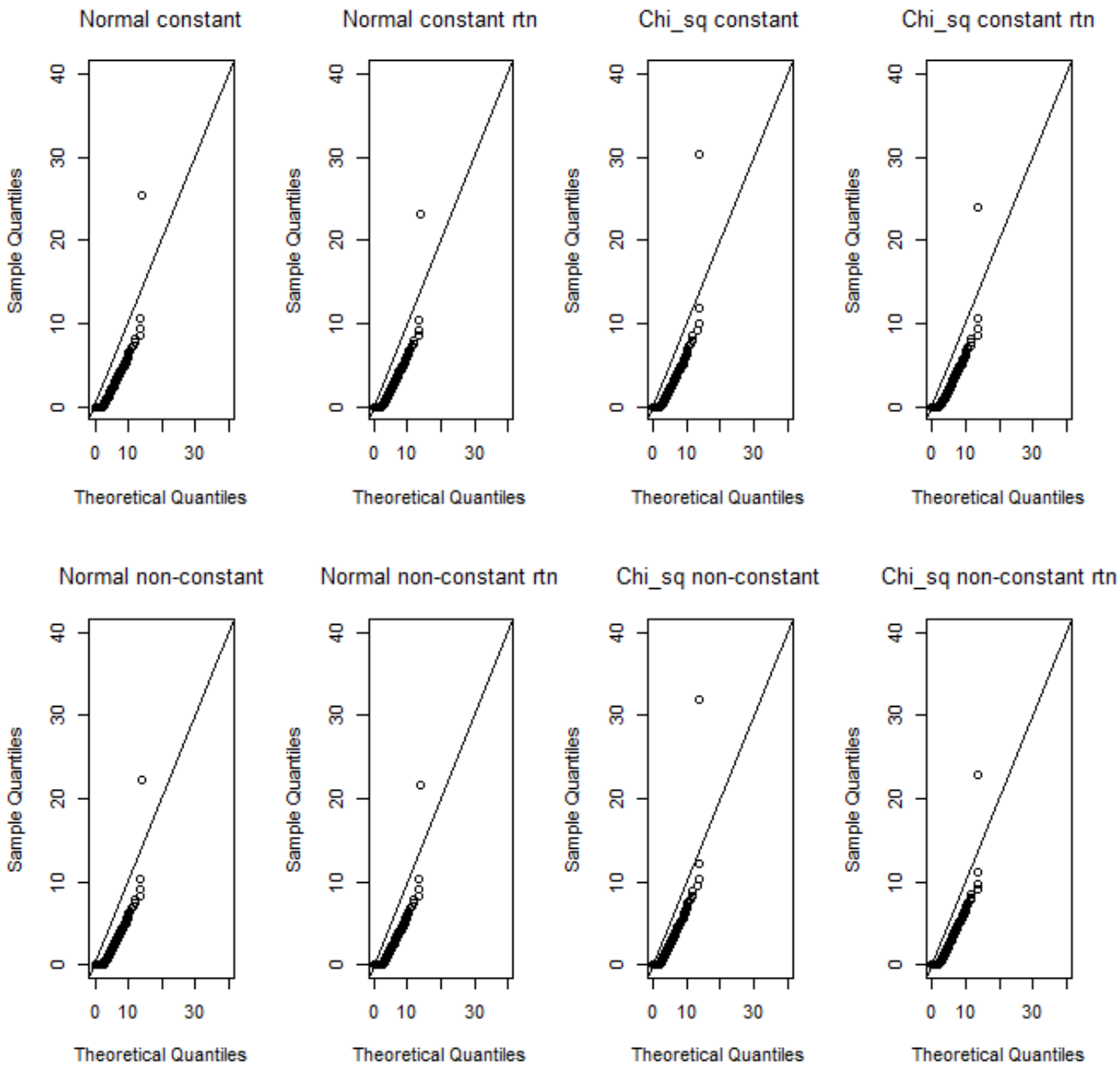

Figure 3. 5 Qq-plots of squared Student's t- test values for association between the multi-locus genotype combination cell 2-2 versus the pooled remaining multi-locus genotypes, for normal and chi-squared trait distributions or non-transformed and rank-transformed to normal data.

Legend Each time, one replicate with epistatic variance, 10% is considered and F-statistics are pooled for all SNP pairs over the 999 permutations. A theoretical F-distribution according to F(1,498) is taken as the reference.

3.3.2 Familywise error rates and false positive rates

Table 3.1 and Table 3.2 report the familywise error rates (FWER) corresponding to the simulation scenario σ_g^2=0 (no epistasis, no main effects) and false positive rates corresponding to σ_g^2=0.05 and 0.1 (scenarios of epistasis in the absence of main effects). We observe that, irrespective of the original trait distribution and whether or not a data transformation preceded MB-MDR analysis, the estimated rates satisfy Bradley's [30] liberal criterion of robustness for the significance level α=0.05 . This criterion requires that the error rates are controlled for any level α of significance, if the empirical rate $\hat{\alpha}$ is contained in the interval $0.5\alpha \le \hat{\alpha} \le 1.5\alpha$.

Table 3. 1 Type I error rates for data generated under the null hypothesis of no genetic association.

TRAIT STATUS		**Familywise error rate (Type I)**							
Distributions	**Variances**	**ST**	**WT**	**Rank_ST**	**Rank_WT**	**Log_ST**	**Log_WT**	**Rtn_ST**	**Rtn_WT**
Normal	Equal	0.040	0.053	0.049	0.049	0.044	0.051	0.050	0.058
Normal	Unequal	0.058	0.066	0.044	0.051	0.064	0.056	0.053	0.058
Chi-square	Equal	0.045	0.036	0.052	0.051	0.055	0.038	0.058	0.056
Chi-square	Unequal	0.053	0.057	0.048	0.052	0.051	0.054	0.043	0.047
t-distribution	Equal	0.048	0.053	0.050	0.059	0.049	0.056	0.052	0.057
t-distribution	Unequal	0.057	0.044	0.042	0.051	0.053	0.048	0.045	0.039

ST-Student's *t*-test, WT-Welch's *t*-test, Rtn- Rank transformation to normal

Table 3. 2 False positive percentages of MB-MDR involving pairs other than the interacting pair (SNP1, SNP2).

	TRAIT STATUS		**FALSE POSITIVES**							
σ_g^2	**Distributions**	**Variances**	**ST**	**WT**	**Rank_ST**	**Rank_WT**	**Log_ST**	**Log_WT**	**Rtn_ST**	**Rtn_WT**
0.05	Normal	Equal	0.040	0.047	0.053	0.048	0.051	0.047	0.050	0.051
	Normal	Unequal	0.051	0.060	0.044	0.061	0.052	0.065	0.048	0.068
	Chi-square	Equal	0.037	0.056	0.051	0.053	0.042	0.054	0.045	0.056
	Chi-square	Unequal	0.040	0.055	0.047	0.042	0.042	0.053	0.047	0.052
	t-distribution	Equal	0.051	0.048	0.048	0.051	0.047	0.047	0.047	0.033
	t-distribution	Unequal	0.053	0.047	0.058	0.057	0.054	0.048	0.051	0.052
0.1	Normal	Equal	0.040	0.067	0.058	0.058	0.053	0.061	0.054	0.063
	Normal	Unequal	0.050	0.065	0.044	0.058	0.048	0.063	0.045	0.057
	Chi-square	Equal	0.048	0.059	0.061	0.060	0.053	0.055	0.057	0.056
	Chi-square	Unequal	0.063	0.041	0.051	0.041	0.061	0.040	0.053	0.036
	t-distribution	Equal	0.048	0.053	0.047	0.049	0.050	0.054	0.044	0.051
	t-distribution	Unequal	0.033	0.050	0.055	0.059	0.036	0.051	0.037	0.051

False positive percentage is defined as the proportion of simulation samples for which at least one pair other than the causal pair (SNP1, SNP2) are significant.

ST-Student's *t*-test, WT-Welch's *t*-test, Rtn- Rank transformation to normal

3.3.3 Empirical power estimates

MB-MDR empirical power estimates for correctly identifying the causal epistatic SNP are given in Table 3.3. For all scenarios higher MB-MDR power is achieved with increasing σ_g^2, i.e., with increasing proportion of epistatic variance to total trait variance. MB-MDR analysis with Welch's *t*-test has generally lower power than MB-MDR with the Student's *t*-test. This power loss is most severe for normal data. A (moderate) power gain is observed for settings where traits are *t*-distributed, variance homogeneity is present, epistatic variance is 10% and data are either left untransformed or are log-transformed prior to MB-MDR analysis. Parametric Student's *t*-tests with the original trait measurements lead to reduced overall MB-MDR power when trait distributions deviate from normality. For non-normally distributed traits, there is a tendency for MB-MDR with Student's *t* applied to rank-transformed data to outperform other MB-MDR analysis approaches (this is: association tests other than Student's *t* and other types of transformation, or no transformation at all). A worthy competitor is MB-MDR with Student's *t* after rank-transforming original traits to normality. The differences in power performance between MB-MDR using untransformed traits or transformed traits are the largest for rank-based transformations compared to logarithmic transformations. No significant differences are observed between empirical power estimates derived from MB-MDR analysis on untransformed traits compared to those analyses based on trait standardization transformations (results not shown).

A graphical representation of the 1000 MB-MDR epistasis test results for the causal SNP pair (*p*-values, multiple testing corrected, as outputted by the MB-MDR software), one for each data set generated under a particular simulation setting (in particular, σ_g^2 = 10%), is given in Figure 3.6. Here, MB-MDR with Student's *t* is considered. Results are depicted for scenarios where the original trait data are derived from a normal (symmetric) or from a chi-squared (non-symmetric) distribution, and then subjected to different data transformation strategies. The scatter plot matrices of Figure 3.6 suggest a tendency for smaller MB-MDR *p*-values to be generated after rank-based data transformations compared to other type of transformations, including the identity transformation (see for instance Panels A and B for normally distributed traits). This tendency becomes more extreme for chi-square distributed traits with non-equal variance (Panel D). Here, it becomes apparent that rank-transformation generally leads to larger *p*-values as compared to rank-transformations to normality. For settings where traits are chi-squared distributed and variance homogeneity is present, the scatter plots of Figure 3.6

(Panel C) are in agreement with the corresponding results in Table 3.3 (power estimates of 100% in the event of a non-identity transformation compared to 90% for MB-MDR applied to untransformed traits). If there were no differences between the untransformed and transformed trait results, we would expect all the points to lie along the diagonal.

Table 3. 3 Power estimates of MB-MDR to detect the correct interacting pair (SNP1, SNP2).

σ_g^2	**TRAIT STATUS**		**POWER**							
	Distributions	**Variances**	**ST**	**WT**	**Rank_ST**	**Rank_WT**	**Log_ST**	**Log_WT**	**Rtn_ST**	**Rtn_WT**
0.05	Normal	Equal	0.400	0.046	0.367	0.001	0.377	0.039	0.378	0.041
	Normal	Unequal	0.330	0.083	0.391	0.001	0.331	0.069	0.344	0.051
	Chi-square	Equal	0.221	0.000	0.953	0.130	0.929	0.466	0.978	0.802
	Chi-square	Unequal	0.317	0.005	0.511	0.002	0.402	0.012	0.578	0.135
	t-distribution	Equal	0.344	0.239	0.920	0.042	0.338	0.240	0.806	0.320
	t-distribution	Unequal	0.383	0.116	0.615	0.002	0.380	0.122	0.543	0.132
0.1	Normal	Equal	0.950	0.634	0.952	0.087	0.959	0.626	0.958	0.650
	Normal	Unequal	0.963	0.743	0.975	0.152	0.955	0.727	0.959	0.690
	Chi-square	Equal	0.897	0.126	1.000	0.922	1.000	1.000	1.000	1.000
	Chi-square	Unequal	0.938	0.350	0.989	0.255	0.975	0.548	0.991	0.884
	t-distribution	Equal	0.873	0.881	1.000	0.885	0.853	0.876	0.999	0.987
	t-distribution	Unequal	0.921	0.801	0.995	0.409	0.921	0.806	0.989	0.834

Power is defined as the proportion of simulated samples of which the causal pair (SNP1, SNP2) is significant.

ST-Student's *t*-test, WT-Welch's *t*-test, Rtn- Rank transformation to normal

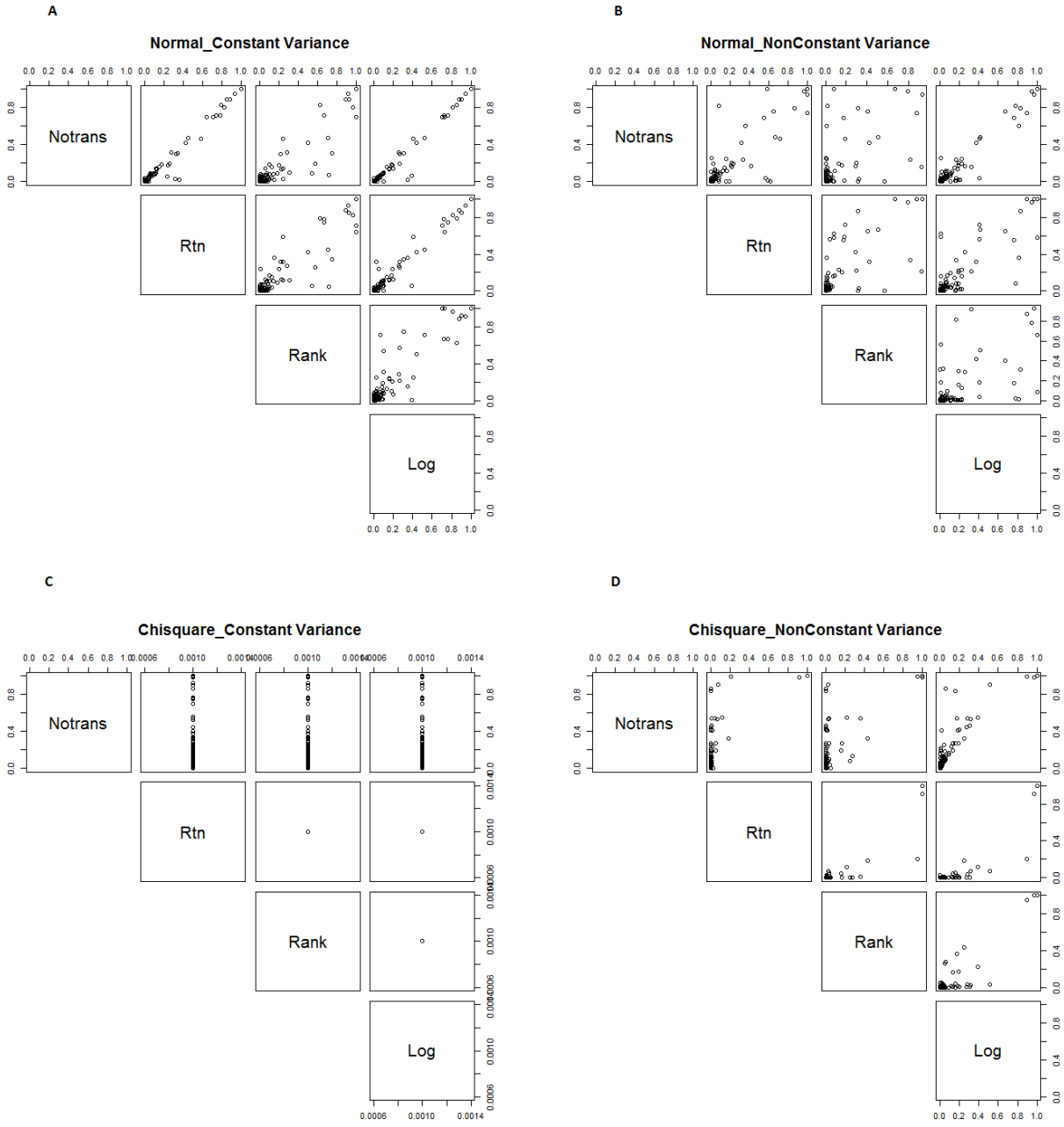

Figure 3. 6 Scatter plot matrices of MB-MDR multiple testing corrected p-values for the causal SNP pair for a variety of a priori data transformations.

Legend Only MB-MDR results with Student's t testing for associations are shown. The epistatic contribution to the trait variance is set to 10%. Different scenario's of trait distribution are considered: normal traits and homogeneity (panel A); normal traits and heteroscedasticity (panel B); chi-squared distributed traits and homogeneity (panel C); chi-squared distributed traits and variance heterogeneity (panel D).

3.4 Discussion

Proposed data mining methods for epistasis detection are seldom thoroughly discussed in terms of their underlying (model) assumptions and their effects on overall power or type I error control. For instance, another well-known data dimensionality reduction method for quantitative traits (generalized MDR - GMDR) [31] is based on score statistics to define differential multilocus genotype groups related to the trait of interest. Although the GMDR method is not necessarily likelihood-based (least-squares regression or other statistical methods for non-normal continuous traits can be employed as well, in theory), continuous phenotypes were only investigated in terms of a normal model, and the software implementation for continuous traits relies on the classical linear regression paradigm to build the score statistics. The authors did not explicitly investigate the power of their method when non-normal continuous data are involved in the context of epistasis screening. Previously, we commented on the advantages and disadvantages of GMDR-like methods compared to MB-MDR (e.g., [28, 32]). Based on these comments, we here focused on MB-MDR while investigating the effects of model-violations on the performance of 2-locus multifactor dimensionality reduction methods for quantitative traits.

For every 2 loci (for 2 bi-allelic SNPs, there are theoretically 9 multilocus genotype combinations), MB-MDR with association *t*-tests subsequently creates two groups, where one group refers to one multilocus genotype and the other to the remaining multilocus genotype combinations. Internally, 2-group comparison tests are performed so as to assign a "label" to each multilocus genotype. This procedure naturally creates highly imbalanced groups, with potentially extreme cases of heteroscedasticity. Although Welch's test is designed to give a valid *t*-test in the presence of different population variances, Welch's *t*-test combined with MB-MDR shows no improved power over the Student's *t*-test for scenarios with unequal variances, even for normally distributed traits (cfr Table 3.3). This can be explained by the fact that the degrees of freedom for the Welch's test become smaller for strongly unequal groups, resulting in a highly conservative test in the event of extreme unbalanced data (see e.g., [33] and Figure 3.1). This motivates our choice to continue working with MB-MDR analyses based on Student's *t* testing to identify groups of multilocus genotypes with differential trait values, despite the underlying trait distribution.

It is well-know that parametric methods have improved statistical power over non-parametric methods when all parametric model assumptions are valid [6, 34]. When an analysis of

residuals detects violations of assumptions of normality and heterogeneity of variance of errors across groups for ANOVA, remedial measures that log-transform the dependent variable and consideration of an ANOVA model assuming unequal variances, may work well. However, in screening settings involving many factors at a time, it is usually impractical to find a single transformation that is universally optimal for all factors. When study data do not meet the distributional assumptions of parametric methods, even after transformation, or when data involve non-interval scale measurements, a non-parametric context is more appropriate. Such a context usually implies testing based on ranks or applying data rank transformations prior to the application of a parametric test.

Strong power increases were observed when data were rank-transformed prior to MB-MDR testing with Student's *t* association testing. This can be understood by noting that the ranks, which are computed for the pooled set of all available quantitative trait measurements, in general reduces the influence of skewness and deviations from normality in the population distribution [35, 36]. The same is achieved by a percentile transformation (Rtn), which – at the same time - preserves the relative magnitude of scores between groups as well as within groups. Only for normally distributed data with equal variances, the ideal scenario for a *t*-test on original traits, a small power loss is observed. Goh and Yap [36] also concluded that rank-based transformation tends to improve power regardless of the distribution. In general, as with traditional two group *t*-testing, deviations from normality seem to be more influential to the power of an MB-MDR analysis with Student's *t* than deviations from homoscedasticity (Table 3.3). This is also in line with the observation that power estimates generally become more optimal for scenarios in which data are transformed to normality prior to MB-MDR analysis compared to scenarios in which they are not. The identical results obtained for untransformed traits and standardized traits (not shown) are not surprising as well. Standardization involves linearly transforming original trait values using the overall trait mean and overall standard deviation. Such a transformation does not affect the two-group *t*-tests within MB-MDR.

Although data transformations are valuable tools, with several benefits, care has to be taken when interpreting results based on transformed data. The inference of epistasis depends upon the scale of measurement in a way that interaction effects can be reduced or eliminated by non-linear monotonic transformations of a dependent variable [37], so also by some rank-based transformations. However, for our simulation scenarios, we have not seen any evidence of such a reduction in interaction signals when using rank-transformed data prior to MB-MDR

analysis (Tables 3.1-3.3, Rank). Application of any epistasis screening tool to real-life data will face the challenge to match observed statistical significance with biological relevance [1].

Despite MB-MDR internal inflations (Figures 3.4 and 3.5), there is no evidence for a cumulative or combined effect on MB-MDR's final results (Tables 3.1 and 3.2), irrespective of the assumed model violation (in terms of deviations from normality or homoscedasticity). This can be explained by the permutation-based step-down maxT approach, which is currently adopted by MB-MDR to correct for multiple testing of SNP pairs.

In many of our practical applications, though we observed a tendency of increased numbers of significant epistasis results with MB-MDR applied to quantitative traits, even after SNP pruning (r^2 below 75%) to avoid potential false positives (or redundant interactions) due to highly correlated SNPs. No such observation was previously made for dichotomous traits. For dichotomous traits, MB-MDR uses a score test, in particular, the Pearson's chi-squared test. This test is known to be affected by unbalanced data, or sparse data, as is the case for rare variants [38]. However, these data artifacts, which question the use of large sample distributions for test statistics, are minimized by requiring a threshold sample size for a multilocus genotype combination, irrespective of whether a dichotomous or quantitative trait is used. Hence, an explanation for the apparent discrepancies between practical and theoretic MB-MDR results may be found in the way the null distribution for multiple testing is derived. It is often forgotten that also permutation-based multiple testing corrective procedures make some assumptions. For instance, for the step-down maxT approach, currently the default multiple testing strategy in MB-MDR, the Family-Wise Error Rate (FWER) is strongly controlled provided the assumption of subset pivotality holds [27]. The subset pivotality ensures that the distribution of any sub-vector of *p*-values does not depend on the truth or falseness of the hypotheses not considered by this sub-vector [39].

Preliminary results on the effect of linkage disequilibrium on MB-MDR error control, as well as on the effect of highly variable minor allele frequencies (and thus highly variable available samples sizes for multilocus genotypes) show that subset pivotality is likely to be violated in real-life settings, giving rise to inflated error rates in the presence of multiple epistasis signals (work in progress). Note that the standard bootstrap method provides the asymptotically correct null distribution for multiple testing and does not require the subset pivotality condition given in Westfall and Young [27]. Resampling-based multiple testing with asymptotic strong control of type I error as corrective method for multiple testing in MB-MDR warrants further investigation.

Summarizing, we observed that non-normally distributed traits can affect the power of final test statistics of MB-MDR with classical *t*-tests for association, and that this influence is primarily driven by the sparser multilocus genotype combinations. Improved power can be obtained by pre-analysis data transformation to normality. MB-MDR permutation-based maxT correction for multiple testing keeps type I error and false positive rates under control, since in all considered simulation scenarios', the assumption of the maxT permutation strategy was plausible.

When performing MB-MDR screening for gene-gene interactions with quantitative traits, we recommend to rank-transform traits to normality prior to MB-MDR analysis with Student's *t* test as preferred association test.

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PART 3

PRACTICAL APPLICATIONS

Analysis 1

Analysis of the High Affinity IgE Receptor Genes Reveals Epistatic Effects of FCER1A Variants on Eczema Risk

Related publication

J. M. Mahachie John*, H. Baurecht*, E. Rodrı´guez, A. Naumann, S. Wagenpfeil, N. Klopp, M. Mempel, N. Novak, T. Bieber, H.E. Wichmann, J. Ring, T. Illig, T. Cattaert, K. Van Steen, S. Weidinger (2010)

**These authors contributed equally.*

*Allergy **65(7)**, 875-882. doi: 10.1111/j.1398-9995.2009.02297.x*

1.1 Aim of the Analysis

We investigated the association of 27 FCER1A, FCER1B (MS4A2), and FCER1G variants with IgE in a large population-based cohort and tested for epistatic effects using the MB-MDR method. In addition, we investigated a potential interaction between 10 FLG and FCER1A variants in a large collection of eczema cases and population controls. We believe that small-scaled genetic studies, based on candidate genes rather than genome-wide data, are useful in methodological development: Due to the complexities of high-dimensional genomic data analysis, both from the biology and statistics perspective, any statistical epistasis detection method is believed to have little viability when not useful on a much smaller scale. Thus, if a method does not work on small-scaled data it will certainly not work on large-scale data sets.

1.2 Data description

KORA S4 is an epidemiological study group including 4261 unrelated German adult individuals representative of the population within the age range of 25–74 years in the city and region of Augsburg (Bavaria, Germany); probands were recruited from 1999 to 2001 [1]. In addition to demographic data, all subjects had to complete a standardized questionnaire that included the basic allergy questions of the European Community Respiratory Health Survey on respiratory health [2]. Total and specific IgE antibodies to aeroallergens (Sx1) were measured using RAST FEIA CAP system (Pharmacia, Freiburg, Germany). Allergic sensitization was defined as specific IgE levels ≥0.35KU/l (CAP class ≥1). DNA was available for 4261 individuals (50.9% females), and all subsequent analyses were based on this number. All German patients with eczema were unrelated and of white origin, with eczema diagnosed on the basis of a skin examination by experienced dermatologists using the UK diagnostic criteria [3]. The collection of 1018 individuals (59.5% females) was recruited in the department of dermatology of the University hospitals of Bonn and Munich.

1.3 MB-MDR Results and Discussion

Results on total IgE indicated strong epistasis between the two FCER1A variants rs2251746 and rs16842010 (*p*-value <0.001). These variants were found to be weakly correlated (r^2 = 0.003) and can thus be regarded as uncorrelated. Taking eczema as outcome, MB-MDR indicated strong epistasis between FLG haploinsufficiency and several SNPs of the FCER1A

gene. Because of their known and confirmed remarkably strong effect on eczema risk, we additionally performed the algorithm with adjustment for FLG effects to determine whether these results were attributed to genuine epistasis. After this correction, the interaction between FLG and FCER1A polymorphisms vanished, but we identified statistical epistasis between the two FCER1A SNPs rs10489854 and rs2511211 (*p*-value =0.046). Concerning linkage disequilibrium, the two variants of the FCER1A genes also showed a weak correlation (r^2 = 0.01). With correction of main effects, no significant results were observed for variants of the FCER1B and FCER1G and FLG genes. In addition, all investigated SNPs were in Hardy-Weinberg Equilibrium. The significant interactions observed from the MB-MDR analysis indicated that these outcome respective two SNPs jointly have a strong effect on total IgE and on eczema.

Analysis 2

Comparison of Genetic Association Strategies in the Presence of Rare Alleles

Related publication

Jestinah M Mahachie John*, Tom Cattaert*, Lizzy De Lobel, François Van Lishout, Alain Empain,Kristel Van Steen (2011)

**These authors contributed equally.*

BMC Proceedings, ***5(Suppl 9):****S32. doi:10.1186/1753-6561-5-S9-S32*

2.1 Aim of the Analysis

We explored the utility of several methods, both parametric and non-parametric, to test for or model genetic associations using population-based and family-based data from Genetic Analysis Workshop 17 (GAW17). Here, we focus on MB-MDR related analyses (MB-MDR-population data and FAM-MDR-family data) incorporating the maxT and minP of Westfall and Young [4] for multiple testing correction.

2.2 Data description

The data provided by GAW17 [5] included a subset of genes grouped according to pathways that had sequence data available in the 1000 Genomes Project. Effect sizes for coding variants within these genes were assigned using PolyPhen and SIFT predictions of the likelihood that the variant would be deleterious. Two hundred replicates were generated. All simulated singular SNP effects (C4S1861, C4S1873, C4S1874, C4S1877, C4S1878, C4S1879, C4S1884, C4S1887, C4S1889, and C4S1890 in the KDR gene and C4S4935 in the VEGFC gene) are assumed to be additive on the quantitative trait scale, such that each copy of the minor allele increases or decreases the mean trait value by an equal amount. The sample size for both population- and family-based data was 697 with family data comprising 8 families with 202 founders and 3 offspring generations. The founders were randomly sampled from the unrelated individuals' data set, and genotypes of offspring were sampled using Mendelian inheritance.

Our analyses involved the quantitative trait *Q1*, which was simulated as a normally distributed phenotype. Furthermore, we restricted attention to the available single-nucleotide polymorphisms (SNPs) on chromosome 4 (944 SNPs). In total, 200 replicates were generated.

2.3 MB-MDR Results and Discussion

We observed that the MB-MDR approach for unrelated individuals had some power (0.14 for max T and 0.34 for min P) to find C4S1878, the marker with the largest MAF (0.16), but also elevated FWER estimates (0.13 and 0.50, respectively). On the other hand, the FAM-MDR approach had not only some power (0.18 for max T and 0.17 for min P) to detect C4S4935 but also kept the FWER under control. The total contribution of markers in linkage disequilibrium with functional markers (r^2>0.9) was only 0.01 to FWER, hence this can be ruled out as an explanation of the increased FWER. Lower MB-MDR power compared to our

MB-MDR analyses on simulation data with normal traits led us to postulate that the rarity of certain marker alleles hampers the validity of model assumptions and distributional properties of test statistics as well as assumptions underlying some commonly used measures to correct for multiple testing or to control false-positive rates.

Analysis 3

Genetic Variation in the Autophagy Gene ULK1 and Risk of Crohn's Disease

Related publication

Liesbet Henckaerts, Isabelle Cleynen, Marko Brinar, Jestinah Mahachie John, Kristel Van Steen, Paul Rutgeerts, and Sèverine Vermeire (2011)

*Inflammatory Bowel Diseases, **17(6)**, 1392-1397. doi: **10**.1002/ibd.21486*

3.1 Aim of the Analysis

We investigated polymorphisms in selected autophagy genes for their association with susceptibility to Crohn's disease. Autophagy has been recently implicated in various human pathological and physiological conditions including cancer, heart diseases, liver disease Crohn's disease etc. The autophagy connection is presented in Figure 3.1.

3.2 Data description

In the framework of the IBD genetics study conducted at the IBD unit of the University Hospital in Leuven, Belgium, we studied 1282 Crohn's disease (CD) patients of Western European origin and a control group of 548 unrelated healthy volunteers without a family history of IBD or other immune-related disorders. Diagnosis of IBD was based on accepted clinical, endoscopic, radiologic, and histologic criteria [7, 8]. As an exploratory cohort, a total of 70 haplotype tagging single nucleotide polymorphisms (tSNPs) in 12 genes were genotyped in a cohort of 947 CD patients and 548 controls. A confirmatory cohort consisting of 335 trios with father, mother, and a child affected with CD was also available. DNA was extracted as described in Miller et al. [9]. Samples and data were stored in a coded and anonymized database.

3.3 MB-MDR Results and Discussion

We found a novel association between one haplotype tagging SNP (rs12303764) in the ULK1 gene and CD. MB-MDR confirmed this association which also was found both in a single SNP analysis and TDT in 335 parent–child CD trios. To further clarify the role of ULK1 in CD, an in-depth investigation of the variation in the region and possible role for copy number variation in this region should be evaluated. No significant epistasis results were observed.

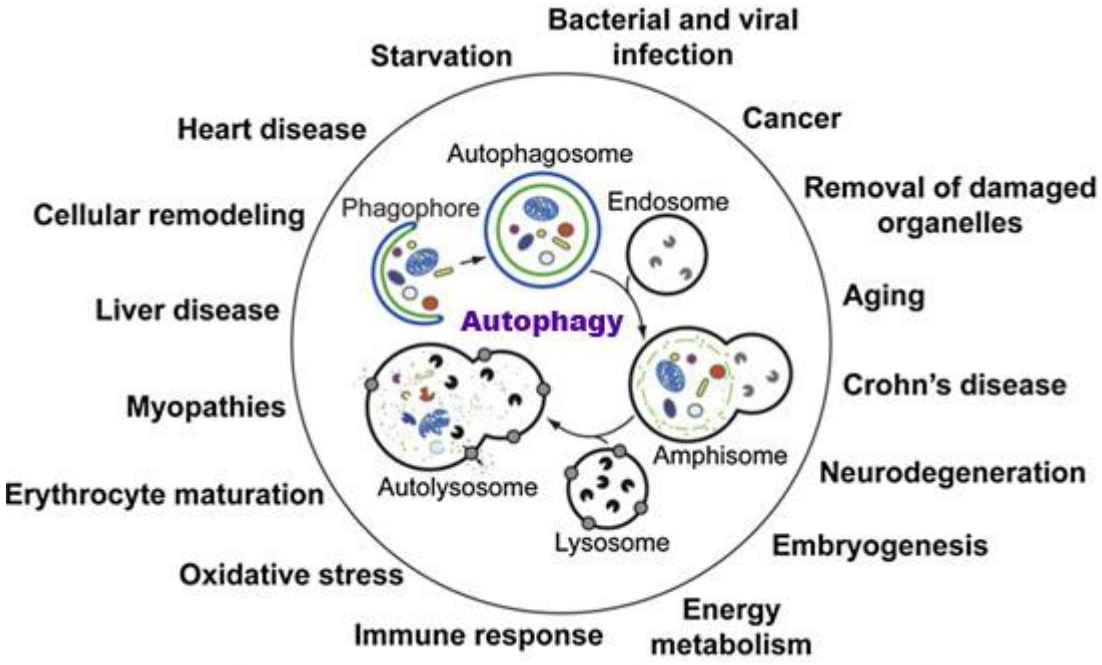

Figure 3.1 The Autophagy Connection. *Source: KLionsky , D J* [10].

Analysis 4

Crohn's Disease Susceptibility Genes Involved in Microbial Sensing, Autophagy and Endoplasmic reticulum (er) Stress and their Interaction

Related publication

Eveline Hoefkens, Jestinah Mahachie John, Kristel Van Steen, Kris Nys, Gert Van Assche, Patrizia Agostinis, Paul Rutgeerts, Séverine Vermeire, and Isabelle Cleynen (2012)

Under Review

4.1 Aim of the Analysis

We investigated association between Chrohn's disease (CD) and 11 SNPs in the autophagy genes *ATG16L1, IRGM* and *MTMR3*; the endoplasmic reticulum (ER) stress gene *ORMDL3*; and the bacterial sensing gene *NOD2* in a large Belgian CD cohort. In addition, we also evaluated these SNPs in a large Belgian Ulcerative Colitis (UC) cohort, to determine whether these variants were specific for CD or also important in UC.

4.2 Data description

The study population consisted of 3451 individuals in total: 1744 CD patients (42% males) and 793 UC patients (55% males) from the IBD unit of the University Hospitals Leuven, and a control group of 914 unrelated healthy controls (48% males). Both patients and controls were of Caucasian origin. Diagnosis of CD or UC was based on accepted clinical, endoscopic, radiologic and histological criteria [7]. Informed consent was obtained from all participants. All DNA samples and data in this study were handled anonymously.

4.3 MB-MDR Results and Discussion

For CD, MB-MDR analysis showed a significant two-way interaction between ATG16L1 and IRGM (*p*-value=0.001): rs2241880 (ATG16L1) – rs10065172 (IRGM). Both of these genes belong to the autophagy pathway with a weak correlation between them, $r^2 = 0.006$. For UC, no significant interactions were observed. Despite the known functional interaction between pathways, we did not find gene-gene interactions between the three pathways of ER stress, autophagy and microbial sensing. We did however found an interaction within the autophagy pathway itself.

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PART 4

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Chapter 1: Discussion

1.1 General objective

The main objective of this thesis was to investigate the performance of Model-Based Multifactor Dimensionality Reduction method for quantitative traits, under a variety of settings and assumptions about the operation epistasis mechanisms.

1.2 General Discussion

Most common complex human diseases are caused by multiple genetic variants. Identifying such genetic variants, as well as the potential modifying effect they have on each other has been and still is a big challenge in genetic epidemiology. Indeed, although genome-wide association (GWA) studies have highlighted many loci associated with common diseases using (mostly) binary or quantitative traits, similar studies targeting gene–gene interactions (epistasis), as in genome-wide association interaction (GWAI) studies have been less successful. Yet, it is believed that epistatic interactions may play a significant role in improving pathogenesis, prevention, diagnosis and treatment of complex human diseases, it needs to be seen how important gene-gene interactions are as a potential source of the so-called *missing heritability* [1]. The limited success of worldwide GWAI studies efforts as compared to GWA studies efforts can be attributed to several factors. A major challenge in analyzing epistasis in GWA studies is the enormous computational demands it involves while analyzing billions of SNP combinations. Whereas many human GWA studies test on the order of one million SNPs (only), considering all pairs of SNPs amounts to approximately 500 billion tests; the number of pairs of SNPs scales quadratically with the number of markers [2]. Due to the large number of interaction tests that need to be performed, in particular, when using traditional statistical testing approaches, searching for epistatic effects poses both computational and statistical challenges. Last but not least, there is a conceptual difficulty related to the success of GWAI studies in that they aim to identify biological mechanisms via mathematical/statistical models; models that may be too simplistic to capture the complexity of the underlying phenomena and are often restricted by scales of measurements.

Having the aforementioned challenges in mind, several methods have been developed to detect (causal) interacting genes. According to Onkamo and Toivonen [3], these methods can be categorized into tree-based methods (e.g, random forests), pattern recognition methods (e.g, support vector machines) or data reduction methods (e.g. model-based dimensionality

reduction). Driven by the popular case-control design, most of the developed methods are restricted to dichotomous traits or at least require categorizing a given quantitative trait into distinct phenotypic categories prior to application. Obviously, often the variability exhibited by many traits fails to fit into distinct discrete phenotypic classes (discontinuous variability), but instead forms a spectrum of phenotypes that blend imperceptively (continuous variability), and this should be acknowledged [4].

We developed a data-mining method, Model-Based Multifactor Dimensionality Reduction (MB-MDR) to detect epistatic interactions under different types of traits [5-7]. MB-MDR is a data mining technique that enables the fast identification of gene-gene interactions among 1000nds of SNPs, without the need to make restrictive assumptions about the genetic modes of inheritance. In this thesis, we zoomed in on curtailing the MB-MDR methodology to quantitative traits and on finding the most optimal conditions and settings for quantitative trait MB-MDR analysis via extensive simulation studies.

Most simulation studies in this thesis involve small data sets in terms of marker design (10 - 100 markers), though the quantitative MB-MDR method applies to GWA data as well. However, given the fact the MB-MDR employs an exhaustive search strategy on the input data, applying the method as such to genomewide SNP data might be infeasible, due to limitations of the available IT environment that may not be able to handle the computational and storage demands a GWAI study induces. To make the MB-MDR methodology less dependent on the properties of the IT environment it operates in, we are developing a protocol for optimal Genome-Wide Association Interaction (GWAI) analysis [8]. This minimal protocol includes input/output data properties and pre-analysis data quality contol procedures (involving Hardy-Weinberg Equilibrium test, marker call rates and marker frequencies) related to GWA analysis in general and GWAI analysis in particular (missing data handling and LD control). The protocol also gives recommendations with respect to integrating available knowledge into the MB-MDR analysis by further reducing the interaction search space, for instance via *Biofilter* [9].

Biofilter explicitly uses biological information about gene-gene relationships and gene-disease relationships to construct multi-SNP models, hereby adopting a gene-centric approach. An upgraded release of Biofilter is expected, and will allow using even a larger amount of available biological and statistical knowledge on gene-gene interactions, for a priori data filtering or a posteriori results interpreting.

It should be noted that pre-filtering can also be done based on statistical knowledge. For instance, Hoh et al. [10] proposed a 2-stage approach in which SNPs that meet some threshold in a test at the first stage are subsequently followed up for modeling interactions at the second stage. Alternatively, Oh et al. [11] developed Gene-MDR. This analysis method is also a 2-stage approach. The first stage, a within-gene analysis, summarises each gene's effect from several SNPs within the same gene. The second stage, the between-gene analysis, involves performing interaction analysis using summarized gene effects derived from stage 1.

Although pre-filtering or multi-stage analyses may come at the cost of losing potentially interesting signals (for instance, failure to detect interactions involving markers without significant marginal effects), in particular prefilitering may be highly beneficial in bridging the gap between statistical and biological or genetical epistasis.

In traditional applications of MDR, model selection within a cross-validation framework is used to select a single best model. This misses other important multilocus models that could be biologically meaningful. Recently, Oki and Motsinger-Reif [12], developed a filter-based approach, using MDR modeling to evaluate and rank (based on classification error) all univariate effects and two-locus epistatic effects hereby prioritizing models for follow-up in replication studies.

In this thesis, we focused on 2-order interactions for bi-allelic SNPs. The MB-MDR software can handle multi-allelic genetic markers and is fairly easily extended to investigate higher-order interactions (i.e. > 2-way interactions) [13]. However, it is questionable whether valid biological interpretations can be given to higher order interactions (>2), when it is already so difficult to do so for order 2. In addition, building sensible animal models to support the statistical findings seems almost untangible. Yet, still emphasizing 2-way genetic interactions, a beta-version of the MB-MDR software allows to tackle research questions such as "How does a fixed (non-) genetic factor (such as smoking or gender) modify the effect of gene-gene interactions?" (see also [14] with smoking as a potential modifying explanatory variable), or phrased differently "Can we observe different gene-gene interaction patterns according to different population subgroups?"

We chose not to include multi-allelic markers in MB-MDR analyses, due to issues related to "sparseness" and "unavailable" multilocus genotypes. These issues require more detailed investigations, as was indicated by our work on epistasis with rare variants. The latter clearly showed that analyzing rare variants with current implementations of MB-MDR highly inflates type I errors [14]. For bi-allelic SNPs, we controlled the sparseness effect on MB-MDR type

I errors by setting a lower bound on the number of individuals required for each multilocus genotype (see Chapter 3 of PART 2). Additional measures are needed when multi-allelic markers or rare variants are involved. In either case, as the aforementioned lower bound in the context of bi-allelic SNPs increases, MB-MDR's type I errors decrease as well (results not shown).

In general, in the case of missing genotypes, the implementation of MB-MDR uses available data. However, MDR and MDR related extensions commonly require complete cases to run the analysis. In order to have complete data, there are mainly two options to follow: 1) simply removing individuals with missing genotypes or 2) imputing missing genotypes. The first option is the least desirable option because it usually leads to invalid analyses when data are not completely missing at random [15], besides throwing away possibly valuable (expensive) information. The second option is to be preferred. The original MDR software adopted a frequency-based imputation strategy to impute missing genotypes with the most common genotype of the respective SNP. Thus, the imputation method is performed for one SNP at the time, prior to MDR analysis. This may be a reasonable option when the extent of missing genotypes is not dramatic, and when missingness occurs at random across cases and controls. Recently, Namkung et al. [16] developed a procedure called 'EM impute' which imputes missing genotypes using the expectation-maximization (EM) algorithm within the MDR process. Thus, unlike the frequency-based imputation, 'EM impute' does not require a separate step of imputation, but rather it imputes missing values within the MDR analysis. 'EM impute' has been made available through the package *imputeMDR* of the R software [17]. Actually, this R package also allows for two other ways of handling missing data: using all available data for given number of SNPs under consideration, or treating missing genotypes as another (usually fourth) genotype category.

The R software also offers a number of other imputation procedures through the R package *imputation* [18]. These and other imputation software tools can obviously also be applied to "complete" data prior to MB-MDR analysis. These tools were primarily developed in the context of biostatistics or GWA studies. We specifically mention the BEAGLE software of Browning and Browning [19], allowing to impute missing ungenotyped markers using a reference panel. This panel may contain data for parent-offspring trios, parent-offspring pairs or unrelated individuals. However, whereas GWA studies exploit LD between markers to find the true causal variants, LD in GWAI studies may induce redundant [20, 21] interactions. (See also following Section 2.2).

The practical application section presented in this thesis identified several significant genetic interacation effects with quantitative MB-MDR that could be confirmed with other statistical methodologies. The concern remains whether these findings can be translated into biologically relevant mechanisms. In the general introduction of this thesis, we discussed the difference between statistical epistasis and biological epistasis, and indicated that a statistically significant interaction is not necessarily biologically significant. This leads into the discussion about replicating epistastic findings. Should a finding be replicated in an independent data set? Is that possible in the context of GWAI studies where large sample sizes are needed and over 50 epistasis [22] models exist for two bi-allelic loci? Should results be confirmed by animal models or human cell cultures? [23] When using the same criteria as for GWA studies, replication in GWAI studies is likely to fail. In our opinion, not being able to replicate epistasis findings in this sense is not the end of the story. As Greene at al. [24] pointed out as well, failure to replicate may provide important clues about 'some hidden' genetic architecture, and it is should be our mission to unravel this architecture.

Chapter 2: Future Perspectives

2.1 Introduction

In this thesis, we presented epistasis screening results of MB-MDR on different scenarios. For all simulated data, we assumed all SNPs to be in Hardy-Weinberg equilibrium and assumed linkage equilibrium between them. In addition, analyses were performed on single traits (univariate phenotypes). However, in reality, markers located on the same chromosome may be in linkage disequilibrium, and when not properly accounted for may lead to spurious results [25]. Moreover, genetic epidemiological studies typically contain data collected on multiple traits that are jointly associated to genes and their interactions. Testing several traits jointly may be more powerful than testing a single trait at a time [26]. Our ongoing and future research activities aim at adjusting for linkage disequilibrium patterns and accommodating multiple traits in MB-MDR epistasis screening. Thus, we intend to assess MB-MDR's flexibility in handling data under linkage disequilibrium and/or data with multiple traits.

2.2 Linkage Disequilibrium

Modeling and detecting gene-gene interactions at multiple quantitative trait loci often assume that the study population is in linkage equilibrium [27]. This assumption is often violated for

real-life GWAI study populations. Genetic association studies owe their success to the ability to detect association signals via markers that are in high linkage disequilibrium (LD) with disease predisposing loci. Indeed, an association between a genetic marker and a trait can be direct (the allele under investigation directly influences the trait) or indirect (the allele is in LD with the disease-predisposing mutation) [28]. The underlying assumption of genetic association studies is that there are some disease causing loci in the genome, and that if the SNPs under investigation and the disease-causing loci are in close proximity, the marker alleles will be associated with the alleles at the disease-causing loci and can be used as proxy to identify the true causal variant [29]. Motsinger et al. [30] showed that strong patterns of LD increase the power of grammatical evolution neural networks (GENN) to detect genetic associations: The higher the LD between the causal variants (involved in an interaction) and their proxy's, the better the chance to find an epistatic association with the trait of interest. In other words, whereas GWA studies exploit strong LD between a genetic marker and causative variant, GWAI studies exploit relation between the causal pair and their proxys' [31].

Little is understood about how to best incorporate LD patterns between genetic markers or genetic markers and true causal variants in epistasis studies, knowing that LD between two genetic markers may induce a 'redundant' epistatic effect of them on the trait under investigation. According to Zhao et al. [32], LD-based measures can serve as useful statistics to detect gene-gene interaction between two unlinked loci. These authors investigated the effect of a variety of LD patterns on the power of an epistasis study, in the presence of gene-gene interactions between two disease-susceptibility loci in Hardy-Weinberg equilibrium and between two unlinked marker loci, each of which in LD with either of two interacting loci. Grady et al. [33] investigated the effect of LD on MDR epistasis screening incoperating varying amounts of LD and different positions of functional loci on a block(s) of LD.

In our future research, we aim at setting up simulations involving varying degrees of LD between genetic markers and investigate the impact of LD within the context of MB-MDR epistasis modeling. This study will enable us to validate the epistasis strategy when imputed data are used, pruned back and then perfom analysis [34].

2.3 MB-MDR for Multivariate Traits

Many complex diseases such as asthma and Crohn's disease, consist of a large number of highly related, rather than independent, clinical or molecular phenotypes.Identification of genetic loci in complex traits has focused largely on one-dimensional genome scans to search

for associations between single markers and the phenotype, despite the fact that multiple trait mapping has proven to be more powerful than single trait mapping in the regression framework [35, 36]. A new technical challenge arises when identifying genetic variations associated simultaneously with correlated traits.

A natural extension of Student's *t* testing (for 2-group comparisons) or ANOVA (for >2 comparisons) is testing based on Hotelling-Lawley's T^2 (short: Hotelling's T^2). Therefore, we developed a multivariate version of the MB-MDR strategy, replacing all univariate t^2 association tests at steps 1 and 2 with their multivariate counterparts, and proposed a discriminant function-based approach to differentiate between *High* and *Low* multilocus genotype cells.

Multivariate MB-MDR can potentially be used to analyze categorical traits as well, by first fitting a multinomial logistic regression model and second considering the multivariate residuals as new traits for MB-MDR. In a pilot study, we applied our multivariate MB-MDR 1D to screen for main effects with a categorical outcome on wheezing, which was defined as a categorical variable with 3 category levels. The data involved 1671 patients from the birth cohort Prevalence and Incidence of Asthma and Mite Allergy (PIAMA) and 101 SNPs. In practice, we fitted a multinomial logistic regression model adjusting for non-genetic confounding factors and took residuals based on this model as the new traits for MB-MDR. Goodness of fit for the residuals was performed via the score test of Goeman and Le Cessie [37]. We then ranked the test results on all SNPs (highest rank translates to lowest *p*-value), per analysis method. This led to a matrix of ranks with rows corresponding to 'observations' (i.e., SNP pairs) and columns referring to 'variables' (i.e., analysis methods). Next, we performed a Principal Components Analysis (PCA) on the results matrix and visualized the output via a biplot (surpressing the observations). The cosine of the angle between two arrows approximates the correlation between the variables they represent. It can be seen from Appendix Figure A4 that the results from a multivariate MB-MDR 1D and the standard analysis method based on multinomial logistic regression (codominant coding) largely agree.

In our future research, we will thoroughly check the flexibility of MB-MDR for multivariate traits to detect epistasis, by setting up simulations and evaluating power and type I error in the same way we did for MB-MDR for univariate traits. One of the challenges is to incooperate lower-order effects adjustments.

2.4 Molecular Reclassification of Cases

Most human diseases have a heterogeneous disorder with differences in severity, location or behavior. The heterogeneity of the disease has important implications towards clinical management, intensity of follow-up, therapy and mode of delivery [38]. For instance in Crohn's disease (CD), patients with a more severe disease course might benefit from early introduction of immunomodulators and/or biologicals, while patients with favorable disease prognosis could be spared from intense treatment and possible side-effects [39]. We recently showed in Cleynen et al. [39] that genetic variants enable the classification of CD patients in distinct clusters (subgroups), which are different from clusters seen in healthy individuals. No significant association between the genetic-based subgroups and a selection of clinical phenotypes was found. Our results indicated that molecular markers show a promising role in disease stratification and pathogenesis.

Some relevant questions in this context still remain: Do the genetically defined clusters in [39] give clues to clinically meaningful subphenotypes of CD? Can we use these subphenotypes as new traits or a multicategorical trait for epistasis screening? Do different subphenotypes lead to or are dictated by different epistatic pattern?

In our future research, we aim to investigate whether interacting loci can contribute as well to identifying sub-phenotypes.

2.5 Population Stratification

Genome-wide association studies have proven to be successful in identifying common SNPs associated with complex and common traits [40, 41]. One of the common problems in population-based GWA studies is population stratification. Several approaches have been used to correct population stratification, including genomic control, structured association, and principal components analysis [42, 43]. In GWA studies, population substructure can be identified through a principal components analysis, which models ancestral genetic differences between cases and controls and then corrects for this in the analysis [43]. If population structure is not accounted for, it can lead to spurious associations GWA studies and in this context several methods have been proposed to deal with this problem [44].

However, as much as population stratification has been investigated in GWA studies, very few or none of the studies have given attention to population stratification in epistasis screening. Spurious epistasis results may also occur in the presence of population

stratification. We performed a preliminary study in which we simulated data to assess the effect of population stratification on epistasis screening using MB-MDR, hereby creating three scenarios'.

Scenario 1: We first generated null data (no population stratificiation, no main effects and no epistasis), with a normally distributed trait. In particular, the data consisted of 100 replicates with 1000 SNPs and 1000 individuals.

Scenario 2: Secondly, we simulated two heterogeneous populations using HAPSIMU, a genetic platform for population based association studies of Zhang et al. [45]. The populations consisted of the western European ancestry (CEPH) and Yoruba from Ibadan (YRI) of Africa. Also these data had 100 replicated sets of 1000 SNPs (999 of which were null SNPs, 1 main effect SNP) and 1000 individuals. In line with the recommendations given in PART 2, Chapter 3, we rank-transformed to normal the traits under study (Rtn). In addition, we derived residuals from a polygenic model for the Rtn traits (Polygenic_Rtn). The traits under investigation (Rtn and Polygenic_Rtn) were additionally permuted in order to remove the main effect and to create the 3rd and last scenario.

Since none of the simulated data involved epistasis (SNP-SNP interaction), any MB-MDR significant result should be labeled as "false positive". MB-MDR results obtained from exhaustive 2-locus epistasis screening showed that type I error was under control for scenario's 1 and 3. Elevated false positive rates were observed for scenario 2 (see Figure A5 in Appendix).

Our next steps will include an in-depth investigation of the effects of population stratification on epistasis screening, and an assessment of different strategies to correct for population stratification in the framework of an MB-MDR analysis. Thus, we will use simulations involving different population structures and will evaluate false positive rates.

2.6 Multiple Testing in MB-MDR Revisited

Clearly, due to the multi-stage nature of the MB-MDR methodology, step 2 MB-MDR test statistics are not F-distributed (even for normal data, and constant variances – see Figure 3.4). Hence, under all circumstances, marginal *p*-values (per two-locus test) need to be derived from permutation based null distributions, after which a multiple testing correction can still take place. There are several multiple testing procedures provided in the *multtest* package in R [46].

In the methodological part of this thesis (Chapter 3), we observed that despite actual deviations from the theoretical distribution of test statistics used in MB-MDR (e.g. Figures 3.3, 3.4 and 3.5), there was no evidence for a negative cumulative or combined effect on MB-MDR's type I errors or false positive rates (Tables 3.1 and 3.2). This can be explained by the fact that significance assessment in MB-MDR was done via the permutation-based step-down maxT approach, which adjusts *p*-values to correct for large-scale multiple testing. It takes into account the joint distribution of the epistasis test statistics and is less conservative than Bonferroni [47], which is highly conservative in the presence of correlated tests (e.g., due to the fact that they involve a common marker, or due to specific LD patterns between markers). Note that our simulations assumed no LD between markers.

In real-life applications, we do not know a priori which nulls are true and which are false. In addition, preliminary results on the effect of linkage disequilibrium on MB-MDR error control, as well as on the effect of highly variable minor allele frequencies (and thus highly variable available samples sizes for multilocus genotypes) show that subset pivotality is likely to be violated in real-life settings, hampering strong control of FWER [47].

Our future steps will involve further investigation of resampling-based multiple testing strategies in conjunction with MB-MDR.

2.7 Increased Efficiency in Lower-Order Effect Correction

In our methodological paper on "Lower-order Effects Adjustment in Quantitative Traits Model-Based Multifactor Dimensionality Reduction" [48], we concluded that always correcting for lower-order effects during epistasis screening should be made standard. The correction for lower-order effects is carried at both steps 1 and 2 of MB-MDR. Correction at the two steps implies longer computation times, since what is done at step 1 in terms of covariates adjustment is repeated at step 2. Computational intensity might seem not to be much for 2-way interactions but increases dramatically for higher-order interactions.

In order to reduce the computational intensity, we assessed correction at step 2 only, using same data with quantitative trait as used before (see PART 2, Chapter 2). The false positives and power profiles under codominant correction are presented in Appendix Figures A6 and A7. The profiles for this correction and the one on both steps are quite similar, with the new implementation being approximately 2.5 times faster. This is a promising result. More work is needed to investigate the pros and cons in full.

In our future research, we aim at rigorously comparing the two correction scenarios under a variety of epistasis models, not only in terms of power performance and false positive behavior, but also in terms of multilocus genotype cell labeling.

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PART 5

CURRICULUM VITAE AND PUBLICATION LIST

Curriculum Vitae

On 02 April 1982, the nation of Zimbabwe was blessed with the birth of a baby girl whom the parents named “Jestinah Mutuku”. Because of the father was filled with joy about this event, he decided to add his own first name to the family name, Mahachie. In the end, the full names of their lovely girl became “Jestinah Mutuku Mahachie John”. Jestinah went through her first education in Zimbabwe and in August 2005, she received her Bachelor of Science Honors degree in Statistics with a first class pass from the Department of Statistics, University of Zimbabwe. From October 2005 to May 2006, Jestinah worked as a researcher at the Rural Electrification Agency (REA). In June 2006, she left REA to become a research assistant at the University of Zimbabwe, department of Human Resources Research Center (HRRC). In the same month Jestinah received good news of her successful application to the Flemish Interuniversity Council in Belgium to pursue a Master’s degree. Hence, from July 2006 to August 2006, Jestinah was already serving her notice period at HRRC. From September 2006 to September 2008, she was enrolled as an International student at the University of Hasselt commonly known as “Universiteit Hasselt”, Belgium. She obtained 2 Master degrees in subsequent years, Master in Applied Statistics with distinction (2007) and Master in Biostatistics with satisfaction (2008). From July 2008 to July 2009, Jestinah worked as biostatistician for the European Network of Excellence on Asthma and Allergy (GA²LEN) affiliated with Ghent University, Belgium. At the same time, in October 2008, Jestinah was already enrolled as a PhD student at the University of Liege, Belgium, of which this thesis is the outcome. Jestinah is married to Genesis Chevure and the two have one handsome boy, Takunda Chevure (born in Belgium on 2 September 2009).

List of Publications as first or contributing author

Impact Factor (IF) source: 2011 Journal Citation Report Science Edition (ISI Web of Knowledge)

Methodological papers

Jestinah M. Mahachie John,Tom Cattaert, François Van Lishout, Kristel Van Steen: Lower-order Effects Adjustment in Quantitative Traits Model-Based Multifactor Dimensionality Reduction (2012), *PLoS ONE*,**7(1)**: e29594.doi: 10.1371/journal.pone.0029594: ***IF = 4.092***

Jestinah M. Mahachie John, François Van Lishout, Kristel Van Steen: Model-Based Multifactor Dimensionality Reduction to Detect Epistasis for Quantitative Traits in the Presence of Error-free and Noisy data (2011), *European Journal of Human Genetics*, **19(6),** 696-703. doi:10.1038/ejhg.2011.17: ***IF = 4.400***

Jestinah M Mahachie John, Tom Cattaert, Lizzy De Lobel, François Van Lishout, Alain Empain, Kristel Van Steen: Comparison of Genetic Association Strategies in the Presence of Rare Alleles (2011), *BMC Proceedings*, **5(Suppl 9):**S32. doi:10.1186/1753-6561-5-S9-S32: ***no Impact Factor***

Cattaert Tom, Calle Luz M, Dudek Scott T, **Mahachie John Jestinah**, Van Lishout François, Urrea Victor, Ritchie Marylyn D: A detailed view on Model-Based Multifactor Dimensionality Reduction for Detecting Gene-gene Interactions in Case-control Data in the Absence and Presence of Noise (2011), *Annals of Human Genetics*, **75(1)**: 78–89. doi: 10.1111/j.1469-1809.2010.00604.x: ***IF = 2.565***

Tom Cattaert, Vı´ctor Urrea, Adam C. Naj, Lizzy De Lobel, Vanessa De Wit, Mao Fu, **Jestinah M.Mahachie John**, Haiqing Shen, M. Luz Calle, Marylyn D. Ritchie, Todd L. Edwards, Kristel Van Steen: FAM-MDR_A Flexible Family-Based Multifactor Dimensionality Reduction Technique to Detect Epistasis Using Related Individuals (2010), *PLoS ONE* **5(4)**: e10304. doi:10.1371/journal.pone.0010304: ***IF = 4.092***

Practical Papers

Inflammatory bowel disease related phenotypes

De Greef E, Hoffman I, D'Haens G, Van Biervliet S, Smets F, Scaillon M, Dewit O, Peeters H, Paquot I, Alliet P, Arts W, Hauser B, Vermeire S, Van Gossum A, Rahier JF, Etienne I, Louis E, Coche JC, **Mahachie John JM**, Van Steen K and Veereman G for the IBD working group of the Belgian Society of Pediatric Gastroenterology, Hepatology and Nutrition (BeSPGHAN) and the Belgian IBD Research and Development Group (BIRD). Safety and Cost of Infliximab for the Treatment of Belgian Pediatric Patients with Crohn's Disease (2012). *Acta Gastroenterologica Belgica - In Press*: ***IF = 0.638***

Liesbet Henckaerts, Isabelle Cleynen, Marko Brinar, **Jestinah Mahachie John**, Kristel Van Steen, Paul Rutgeerts, Séverine Vermeire: Genetic Variation in the Autophagy Gene ULK1 and Risk for Crohn's Disease (2011), *Inflammatory Bowel Diseases*, **17(6)**, 1392-1397. doi: **10**.1002/ibd.21486: ***IF= 4.855***

Matthias Jürgens, **Jestinah M. Mahachie John**, Isabelle Cleynen, Fabian Schnitzler, Herma Fidde, Vera Ballet, Maja Noman, Ilse Hoffman, Gert Van Assche, Paul J. Rutgeerts, Kristel Van Steen, Severine Vermeire: Clinical Usefulness of C-reactive Protein in the Long Term Management of Crohn's Disease Patients Treated with Infliximab (2011), *Clinical gastroenterology and hepatology* (CGH), **9(5)** 421-427.e1: doi:10.1016/j.cgh.2011.02.008: ***IF = 5.627***

Isabelle Cleynen, **Jestinah M. Mahachie John**, Liesbet Henckaerts, Wouter Van Moerkercke, Paul Rutgeerts, Kristel Van Steen, and Severine Vermeire: Molecular Reclassification of Crohn's Disease by Cluster Analysis of Genetic Variants (2010), *PLoS One*, **5(9)**: e12952. doi:10.1371/journal.pone.0012952: ***IF = 4.092***

Asthma, Eczema and Cystic Fibrosis related phenotypes

Karin Lødrup Carlsen; Stephanie Roll; Kai-Håkon Carlsen; Petter Mowinckel; Alet Wijga; Bert Brunekreef; Maties Torrent; Graham Roberts; Hasan Arshad; Inger Kull; Ursula Krämer; Andrea von Berg; Esben Eller; Arne Høst; Claudia Kuehni; Ben Spycher; Jordi Sunyer; Chih-Mei Chen; Andreas Reich; Anna Asarnoj; Carmen Puig; Olf Herbarth; **Jestinah Mahachie John**; Kristel van Steen; Stefan N Willich; Hans Ulrich Wahn; Susanne Lau; Thomas Keil. Pets in Infancy - Asthma or Allergy at School Age? Pooled Analysis of Individual Participant Data from 11 European Birth Cohorts (2012), *PLoS ONE*, **7(8):** e43214. doi: 10.1371/journal.pone.0043214: ***IF = 4.092***

J. M. Mahachie John, H. Baurecht, E. Rodrı´guez, A. Naumann, S. Wagenpfeil, N. Klopp, M. Mempel, N. Novak, T. Bieber, H.-E. Wichmann, J. Ring, T. Illig, T. Cattaert, K. Van Steen, S. Weidinger: Analysis of the High Affinity IgE Receptor Genes Reveals Epistatic Effects of FCER1A Variants on Eczema Risk (2010), *Allergy* **65(7)**, 875-882. doi: 10.1111/j.1398-9995.2009.02297.x: ***IF = 6.271***

Haerynck F, Van Steen K, Cattaert T, Loeys B, Van Daele S, Schelstraete P, Claes K, Van Thielen M, De Canck I, **Mahachie John JM**, De Baets F: Polymorphisms in the Lectin Pathway Genes as a Possible Cause of early Chronic Pseudomonas Aeruginosa Colonization in Cystic Fibrosis Patients (2012), *Hum Immunol*-In Press: 10.1016/j.humimm.2012.08.010: ***IF= 2.837***

Imaging related papers

Jan E. Vandevenne, Filip Vanhoenacker, **Jestinah M. Mahachie John**, Geert Gelin and Paul M. Parizel: Fast MR Arthrography using VIBE Sequences to Evaluate the Rotator Cuff (2009) , Skeletal Radiology, **38 (7)**, 669-674. doi: 10.1007/s00256-009-0677-y: ***IF = 1.541***

Papers under revision, under review, submitted or currently under construction

François Van Lishout, Tom Cattaert, **Jestinah M Mahachie John**, Elena S Gusareva, Victor Urrea, Isabelle Cleynen, Emilie Théâtre, Benoît Charloteaux, M. Luz Calle, Louis Wehenkel and Kristel Van Steen. An Efficient Algorithm to Perform Multiple Testing in Epistasis Screening- *Under Revision*

Jestinah M Mahachie John, Elena Gusareva, François Van Lishout, Kristel Van Steen. A Robustness Study to Investigate the Performance of Parametric and Non-parametric tests used in Model-Based Multifactor Dimensionality Reduction Epistasis Detection- *Under Revision*

Isabelle Cleynen, Emilie Vazeille , Marta Artieda , Hein W Verspaget , Magdalena Szczypiorska , Marie-Agnès Bringer , Peter L Lakatos , Frank Seibold , Kirstie Parnell , Rinse K Weersma , **Jestinah M Mahachie John** , Rebecca Morgan-Walsh , Dominiek Staelens , Ingrid Arijs , Gert De Hertogh , Stefan Müller , Atilla Tordai , Daniel W Hommes , Tariq Ahmad , Cisca Wijmenga , Sylvia L.F. Pender , Paul Rutgeerts , Kristel Van Steen , Daniel Lottaz , Séverine Vermeire , Arlette Darfeuille-Michaud. Genetic and Microbial Factors Modulating the Ubiquitin Proteasome System in Inflammatory Bowel Disease- *Submitted*

E.Hoefkens, **J.M. Mahachie John**, K.Van Steen, K.Nys, G.Van Assche, P.Agostinis, P.Rutgeerts, S.Vermeire and I.Cleynen. Crohn's Disease Susceptibility Genes Involved in Microbial Sensing, Autophagy and Endoplasmic Reticulum (er) Stress and their Interaction- *Submitted*

Florian Beigel Julia Seiderer, Anni Steinborn, Fabian Schnitzler, Cornelia Tillack, Simone Breiteneicher, **Jestinah Mahachie John**, Kristel Van Steen, Burkhard Göke, Stephan Brand, Thomas Ochsenkühn. Increased Risk of Malignancies in IBD Patients Treated with Thiopurines Compared to Anti-TNF-Antibody Treated Patients in a Large Single Center Cohort- *Submitted*

E. De Greef, **J. Mahachie John**, I. Hoffman, F. Smets, S. Van Biervliet, M. Scaillon, B. Hauser , I. Paquot , P. Alliet , W. Arts , O. Dewit, H. Peeters , F. Baert , G. D Haens, J.F. Rahier, I. Etienne, O. Bauraind , A. Van Gossum, S. Vermeire , F. Fontaine, V. Muls, E. Louis, F. Van De mierop , J.C. Coche, K. Van Steen, G. Veereman. Profile of Belgian Pediatric Crohn's Disease (CD) Patients: Associations between Variables at Diagnosis- *Submitted*

E. De Greef, B. Maus, I. Hoffman, F. Smets, S. Van Biervliet, M. Scaillon, B. Hauser , I. Paquot , P. Alliet , W. Arts , O. Dewit, H. Peeters , F. Baert , G. D Haens, J.F. Rahier, I. Etienne, O. Bauraind , A. Van Gossum, S. Vermeire , F. Fontaine, V. Muls, E. Louis, F. Van De mierop , J.C. Coche, **JM Mahachie John**, K. Van Steen, G. Veereman. Diagnosing and Treating Pediatric Crohn's Disease Patients: Is there a Difference between Adult and Pediatric Gastroenterologist's Practices? Results of the Belcro Cohort- *Under construction*

Cleynen Isabelle, Vazeille Emilie, Artieda Marta, Szczypiorska Magdalena, Bringer Marie-Agnès, Verspaget W. Hein, Lakatos L. Peter, Seibold Frank, Ahmad Tariq, Weersma K. Rinse, **Jestinah Mahachie John**, Arijs Ingrid, Müller Stephan, Tordai Atilla, Hommes W. Daniel, Parnell Kirstie, Wijmenga Ciska, Rutgeerts Paul, Lottaz Daniel, Van Steen Kristel, Darfeuille-Michaud Arlette, and Vermeire Severine. Evidence for a Role of the Familial Cylindromatosis Tumor Suppressor CYLD in Crohn's Disease: Results from a European Consortium- *Under construction*

Gusareva ES, **Mahachie John JM**, Isaacs A, Van Steen K. Application of Mixed Polygenic Model to Control for Cryptic/Genuine Relatedness and Population Stratification-*Under construction*

APPENDIX: SUPPLEMENTARY MATERIAL

Table A1 Empirical power of MB-MDR to detect the correct two functional loci, excluding scenarios of simulated genetic heterogeneity

			Model 27			Model 170		
				Main Effects Correction			Main Effects Correction	
p	σ_g^2	**Noisiness**	**No Correction**	**Additive**	**Codominant**	**No Correction**	**Additive**	**Codominant**
0.1	0.01	None	0.446	0.056	0.018	0.542	0.006	0.006
		MG5	0.338	0.018	0.004	0.414	0.014	0.004
		MG10	0.308	0.012	0.004	0.312	0.008	0.000
		GE5	0.180	0.012	0.002	0.194	0.000	0.000
		GE10	0.094	0.000	0.000	0.116	0.000	0.000
		PM25	0.152	0.016	0.010	0.180	0.004	0.002
		PM50	0.034	0.000	0.000	0.028	0.000	0.000
	0.02	None	0.904	0.264	0.146	0.930	0.144	0.072
		MG5	0.862	0.240	0.108	0.908	0.096	0.046
		MG10	0.784	0.190	0.068	0.800	0.070	0.040
		GE5	0.620	0.092	0.030	0.666	0.050	0.004
		GE10	0.366	0.018	0.010	0.444	0.006	0.002
		PM25	0.506	0.084	0.018	0.554	0.010	0.010
		PM50	0.134	0.012	0.006	0.126	0.002	0.000
	0.03	None	0.996	0.592	0.384	0.996	0.392	0.182
		MG5	0.990	0.526	0.272	0.992	0.348	0.182
		MG10	0.960	0.412	0.240	0.976	0.244	0.102
		GE5	0.912	0.232	0.070	0.922	0.136	0.008
		GE10	0.648	0.084	0.060	0.748	0.054	0.002
		PM25	0.834	0.210	0.096	0.856	0.094	0.046
		PM50	0.286	0.038	0.016	0.284	0.006	0.004
	0.05	None	1.000	0.884	0.842	1.000	0.904	0.554
		MG5	1.000	0.850	0.774	1.000	0.790	0.442
		MG10	1.000	0.772	0.684	1.000	0.680	0.370
		GE5	0.994	0.598	0.488	1.000	0.488	0.098
		GE10	0.940	0.272	0.238	0.970	0.198	0.026
		PM25	0.990	0.562	0.346	1.000	0.314	0.168
		PM50	0.598	0.150	0.050	0.664	0.034	0.030
	0.1	None	1.000	1.000	1.000	1.000	1.000	0.958
		MG5	1.000	0.992	0.998	1.000	1.000	0.928
		MG10	1.000	0.990	0.994	1.000	0.996	0.844
		GE5	1.000	0.978	0.978	1.000	0.982	0.638
		GE10	1.000	0.876	0.874	1.000	0.750	0.310
		PM25	1.000	0.904	0.874	1.000	0.942	0.662
		PM50	0.950	0.478	0.266	0.986	0.220	0.114

Table A1 Continued

			Model 27			Model 170		
				Main Effects Correction			Main Effects Correction	
p	σ_g^2	**Noisiness**	**No Correction**	**Additive**	**Codominant**	**No Correction**	**Additive**	**Codominant**
0.25	0.01	None	0.424	0.038	0.024	0.376	0.300	0.180
		MG5	0.340	0.042	0.030	0.332	0.266	0.154
		MG10	0.284	0.018	0.010	0.248	0.184	0.082
		GE5	0.298	0.012	0.010	0.232	0.184	0.084
		GE10	0.198	0.018	0.006	0.084	0.070	0.032
		PM25	0.146	0.010	0.000	0.144	0.112	0.048
		PM50	0.024	0.002	0.000	0.026	0.014	0.006
	0.02	None	0.912	0.178	0.138	0.908	0.864	0.710
		MG5	0.836	0.150	0.108	0.846	0.780	0.616
		MG10	0.780	0.130	0.104	0.766	0.714	0.532
		GE5	0.840	0.136	0.088	0.674	0.626	0.440
		GE10	0.696	0.078	0.050	0.428	0.342	0.210
		PM25	0.480	0.046	0.040	0.440	0.384	0.250
		PM50	0.116	0.012	0.006	0.110	0.080	0.040
	0.03	None	0.998	0.500	0.444	0.992	0.990	0.952
		MG5	0.984	0.426	0.356	0.992	0.984	0.936
		MG10	0.970	0.306	0.252	0.972	0.960	0.856
		GE5	0.980	0.310	0.242	0.922	0.886	0.752
		GE10	0.930	0.212	0.130	0.728	0.668	0.484
		PM25	0.796	0.122	0.096	0.788	0.722	0.546
		PM50	0.224	0.016	0.006	0.234	0.182	0.090
	0.05	None	1.000	0.878	0.838	1.000	1.000	1.000
		MG5	1.000	0.822	0.784	1.000	1.000	1.000
		MG10	1.000	0.740	0.678	1.000	1.000	0.998
		GE5	1.000	0.730	0.630	0.998	1.000	0.994
		GE10	1.000	0.546	0.384	0.978	0.964	0.906
		PM25	0.992	0.434	0.362	0.990	0.978	0.934
		PM50	0.580	0.078	0.054	0.588	0.528	0.328
	0.1	None	1.000	1.000	1.000	1.000	1.000	1.000
		MG5	1.000	1.000	1.000	1.000	1.000	1.000
		MG10	1.000	0.996	0.996	1.000	1.000	1.000
		GE5	1.000	1.000	0.992	1.000	1.000	1.000
		GE10	1.000	0.986	0.982	1.000	1.000	1.000
		PM25	1.000	0.960	0.932	1.000	1.000	1.000
		PM50	0.970	0.342	0.288	0.970	0.954	0.878

Table A1 Continued

			Model 27			Model 170		
				Main Effects Correction			Main Effects Correction	
p	σ_g^2	**Noisiness**	**No Correction**	**Additive**	**Codominant**	**No Correction**	**Additive**	**Codominant**
0.5	0.01	None	0.386	0.050	0.000	0.332	0.256	0.206
		MG5	0.306	0.034	0.002	0.284	0.206	0.152
		MG10	0.222	0.024	0.000	0.252	0.168	0.124
		GE5	0.272	0.022	0.000	0.188	0.128	0.096
		GE10	0.260	0.015	0.000	0.116	0.068	0.028
		PM25	0.140	0.020	0.002	0.100	0.058	0.032
		PM50	0.034	0.006	0.000	0.024	0.012	0.008
	0.02	None	0.848	0.200	0.000	0.850	0.798	0.770
		MG5	0.780	0.152	0.000	0.792	0.728	0.668
		MG10	0.732	0.110	0.000	0.700	0.632	0.606
		GE5	0.782	0.132	0.000	0.612	0.540	0.454
		GE10	0.740	0.092	0.000	0.374	0.284	0.212
		PM25	0.400	0.044	0.002	0.426	0.346	0.288
		PM50	0.098	0.012	0.000	0.088	0.052	0.036
	0.03	None	0.978	0.460	0.016	0.984	0.988	0.978
		MG5	0.978	0.354	0.004	0.974	0.960	0.948
		MG10	0.926	0.266	0.000	0.942	0.910	0.898
		GE5	0.966	0.344	0.004	0.898	0.860	0.832
		GE10	0.956	0.246	0.002	0.680	0.616	0.516
		PM25	0.704	0.116	0.000	0.780	0.688	0.618
		PM50	0.224	0.020	0.000	0.200	0.138	0.098
	0.05	None	1.000	0.832	0.034	1.000	1.000	1.000
		MG5	1.000	0.768	0.026	0.998	0.998	0.998
		MG10	1.000	0.714	0.024	0.998	1.000	1.000
		GE5	1.000	0.738	0.012	0.998	1.000	0.998
		GE10	0.998	0.658	0.006	0.958	0.948	0.916
		PM25	0.982	0.366	0.008	0.968	0.952	0.950
		PM50	0.514	0.078	0.000	0.466	0.358	0.294
	0.1	None	1.000	0.998	0.316	1.000	1.000	1.000
		MG5	1.000	0.998	0.286	1.000	1.000	1.000
		MG10	1.000	0.994	0.168	1.000	1.000	1.000
		GE5	1.000	0.998	0.266	1.000	1.000	1.000
		GE10	1.000	0.988	0.142	1.000	1.000	1.000
		PM25	1.000	0.902	0.052	1.000	1.000	1.000
		PM50	0.946	0.338	0.004	0.950	0.928	0.912

Table A2 False positive percentage of analyses with identified significant epistasis models other than the correct two functional interacting loci, in the absence of GH.

			Model 27			Model 170		
				Main Effects Correction			Main Effects Correction	
p	σ_g^2	**Noisiness**	**No Correction**	**Additive**	**Codominant**	**No Correction**	**Additive**	**Codominant**
0.1	0.01	None	**0.148**	0.038	0.034	**0.278**	**0.062**	**0.056**
		MG5	**0.124**	0.046	0.044	**0.216**	0.046	0.040
		MG10	**0.126**	0.050	0.044	**0.200**	0.034	0.032
		GE5	**0.114**	0.046	0.044	**0.146**	0.046	0.044
		GE10	**0.102**	0.048	0.038	**0.154**	**0.058**	0.048
		PM25	**0.082**	0.050	**0.054**	**0.142**	0.038	0.040
		PM50	**0.064**	0.046	0.046	**0.086**	0.044	0.038
	0.02	None	**0.282**	0.048	0.044	**0.556**	**0.054**	0.032
		MG5	**0.290**	0.048	0.038	**0.534**	0.034	0.020
		MG10	**0.242**	0.040	0.030	**0.470**	**0.054**	0.040
		GE5	**0.224**	0.034	0.038	**0.374**	0.050	0.034
		GE10	**0.182**	0.038	0.032	**0.286**	0.028	0.030
		PM25	**0.156**	0.042	0.038	**0.286**	0.044	0.044
		PM50	**0.084**	0.044	0.034	**0.120**	**0.052**	0.046
	0.03	None	**0.534**	0.042	0.032	**0.792**	**0.092**	**0.052**
		MG5	**0.436**	0.026	0.024	**0.766**	0.038	0.024
		MG10	**0.432**	0.046	0.042	**0.742**	**0.088**	**0.066**
		GE5	**0.338**	**0.058**	0.050	**0.608**	**0.052**	0.036
		GE10	**0.232**	0.034	0.030	**0.436**	0.042	0.032
		PM25	**0.240**	0.042	0.034	**0.434**	**0.054**	0.038
		PM50	**0.122**	0.044	0.044	**0.182**	0.050	0.042
	0.05	None	**0.754**	0.036	0.034	**0.986**	**0.160**	0.038
		MG5	**0.750**	0.022	0.018	**0.988**	**0.100**	0.030
		MG10	**0.694**	0.048	0.034	**0.970**	**0.096**	0.038
		GE5	**0.682**	**0.054**	**0.056**	**0.920**	**0.062**	0.032
		GE10	**0.514**	0.034	0.022	**0.754**	**0.076**	**0.062**
		PM25	**0.480**	0.050	0.040	**0.776**	**0.080**	0.038
		PM50	**0.184**	0.048	0.038	**0.288**	**0.060**	0.042
	0.1	None	**0.990**	**0.072**	**0.056**	**1.000**	**0.394**	0.050
		MG5	**0.988**	0.050	0.030	**1.000**	**0.340**	0.030
		MG10	**0.970**	**0.066**	0.048	**1.000**	**0.302**	0.042
		GE5	**0.944**	**0.068**	0.050	**1.000**	**0.172**	0.044
		GE10	**0.884**	0.050	0.036	**0.996**	**0.078**	0.030
		PM25	**0.822**	0.050	0.036	**0.992**	**0.158**	0.038
		PM50	**0.404**	0.034	0.036	**0.690**	**0.060**	0.036

Estimates exceeding 0.05 are highlighted in **bold**.

Table A2 Continued

			Model 27			Model 170		
				Main Effects Correction			Main Effects Correction	
p	σ_g^2	**Noisiness**	**No Correction**	**Additive**	**Codominant**	**No Correction**	**Additive**	**Codominant**
0.25	0.01	None	**0.260**	0.044	0.034	**0.060**	0.050	0.026
		MG5	**0.212**	0.044	0.036	**0.074**	**0.056**	0.048
		MG10	**0.218**	**0.060**	0.048	**0.076**	**0.070**	0.048
		GE5	**0.250**	**0.060**	**0.052**	**0.088**	**0.066**	**0.060**
		GE10	**0.210**	0.032	0.026	**0.052**	0.038	0.042
		PM25	**0.158**	0.042	0.044	**0.060**	0.046	0.038
		PM50	**0.082**	0.048	0.048	**0.072**	0.044	0.038
	0.02	None	**0.556**	0.048	0.032	**0.108**	0.048	0.034
		MG5	**0.522**	0.050	0.038	**0.092**	**0.058**	0.042
		MG10	**0.534**	0.050	0.036	**0.092**	**0.058**	0.044
		GE5	**0.518**	**0.066**	0.046	**0.094**	**0.052**	0.040
		GE10	**0.452**	**0.066**	**0.056**	**0.080**	0.044	0.032
		PM25	**0.268**	**0.056**	0.040	**0.076**	**0.052**	0.040
		PM50	**0.128**	0.040	0.030	**0.054**	0.048	0.032
	0.03	None	**0.850**	**0.064**	0.028	**0.122**	**0.056**	0.034
		MG5	**0.786**	**0.054**	0.028	**0.134**	**0.056**	0.042
		MG10	**0.728**	**0.052**	0.040	**0.110**	**0.054**	0.044
		GE5	**0.738**	**0.072**	0.036	**0.106**	0.048	0.030
		GE10	**0.694**	0.050	0.026	**0.120**	**0.056**	0.030
		PM25	**0.492**	0.018	0.018	**0.074**	0.038	0.028
		PM50	**0.198**	**0.058**	0.042	**0.064**	0.040	0.032
	0.05	None	**0.992**	**0.098**	0.034	**0.242**	**0.082**	0.044
		MG5	**0.976**	**0.078**	0.032	**0.208**	**0.062**	0.028
		MG10	**0.964**	**0.078**	0.038	**0.190**	**0.064**	0.034
		GE5	**0.984**	**0.076**	0.024	**0.206**	**0.070**	0.034
		GE10	**0.934**	**0.066**	0.020	**0.176**	**0.056**	0.038
		PM25	**0.786**	**0.054**	0.026	**0.132**	**0.058**	0.034
		PM50	**0.354**	**0.062**	0.046	**0.086**	0.046	0.040
	0.1	None	**1.000**	**0.212**	0.028	**0.528**	**0.120**	0.032
		MG5	**1.000**	**0.196**	0.040	**0.502**	**0.110**	0.032
		MG10	**1.000**	**0.178**	0.036	**0.462**	**0.078**	0.022
		GE5	**1.000**	**0.216**	0.040	**0.412**	**0.112**	0.036
		GE10	**0.998**	**0.154**	0.040	**0.358**	**0.088**	0.040
		PM25	**0.992**	**0.108**	0.044	**0.286**	**0.090**	0.032
		PM50	**0.748**	**0.052**	0.028	**0.122**	**0.058**	0.042

Table A2 Continued

			Model 27			Model 170		
				Main Effects Correction			Main Effects Correction	
p	σ_g^2	**Noisiness**	**No Correction**	**Additive**	**Codominant**	**No Correction**	**Additive**	**Codominant**
0.5	0.01	None	**0.378**	**0.088**	0.022	**0.056**	0.046	0.036
		MG5	**0.324**	**0.056**	0.022	0.044	0.034	0.024
		MG10	**0.308**	**0.066**	0.026	**0.058**	**0.068**	0.048
		GE5	**0.362**	**0.066**	0.030	**0.058**	0.048	0.042
		GE10	**0.322**	**0.077**	0.026	0.038	0.022	0.014
		PM25	**0.210**	**0.084**	0.036	0.044	0.032	0.034
		PM50	**0.112**	**0.052**	0.036	0.046	0.038	0.028
	0.02	None	**0.772**	**0.216**	0.036	**0.074**	**0.054**	0.044
		MG5	**0.750**	**0.168**	0.026	**0.052**	0.032	0.030
		MG10	**0.692**	**0.130**	0.028	0.044	0.050	0.038
		GE5	**0.744**	**0.140**	0.036	0.032	0.026	0.022
		GE10	**0.684**	**0.130**	0.038	**0.054**	0.042	0.032
		PM25	**0.420**	**0.100**	0.036	0.050	0.044	0.034
		PM50	**0.160**	**0.062**	0.034	**0.064**	**0.052**	**0.054**
	0.03	None	**0.954**	**0.376**	0.042	0.044	0.034	0.028
		MG5	**0.948**	**0.284**	0.046	**0.060**	0.042	0.036
		MG10	**0.910**	**0.242**	**0.054**	**0.064**	0.030	0.028
		GE5	**0.924**	**0.270**	0.038	**0.052**	0.034	0.030
		GE10	**0.912**	**0.224**	0.032	**0.052**	0.038	0.034
		PM25	**0.648**	**0.154**	0.026	0.046	0.038	0.032
		PM50	**0.270**	**0.088**	0.044	0.044	0.040	0.030
	0.05	None	**0.998**	**0.608**	0.028	**0.058**	**0.056**	0.040
		MG5	**1.000**	**0.576**	0.036	0.042	0.032	0.024
		MG10	**0.998**	**0.522**	0.042	0.038	0.028	0.036
		GE5	**1.000**	**0.594**	0.042	**0.060**	0.042	0.030
		GE10	**1.000**	**0.500**	0.038	0.034	0.038	0.036
		PM25	**0.968**	**0.314**	0.042	0.048	0.042	0.036
		PM50	**0.458**	**0.124**	0.040	0.036	0.032	0.026
	0.1	None	**1.000**	**0.978**	0.028	**0.056**	0.038	0.030
		MG5	**1.000**	**0.956**	0.036	**0.054**	0.046	0.034
		MG10	**1.000**	**0.928**	0.040	0.048	0.044	0.040
		GE5	**1.000**	**0.982**	0.024	**0.052**	0.040	0.028
		GE10	**1.000**	**0.912**	0.032	**0.054**	0.038	0.028
		PM25	**1.000**	**0.732**	0.026	0.050	0.036	0.030
		PM50	**0.884**	**0.288**	0.034	0.036	0.028	0.028

Table A3 Empirical variance decomposition for model M170, in the absence of GH.

p	σ_g^2	**Noisiness**	σ_{add}^2	$\sigma_{add}^2/\sigma_{main}^2$	σ_{main}^2	$\sigma_{main}^2/\sigma_{gen}^2$	σ_{epi}^2	$\sigma_{epi}^2/\sigma_{gen}^2$	σ_{gen}^2
0.1	0.01	None	0.005	0.803	0.006	0.592	0.004	0.408	0.011
		MG5	0.005	0.755	0.006	0.576	0.004	0.424	0.010
		MG10	0.005	0.820	0.006	0.575	0.004	0.425	0.010
		GE5	0.003	0.828	0.004	0.633	0.002	0.367	0.006
		GE10	0.003	0.910	0.003	0.702	0.001	0.298	0.005
		PM25	0.003	0.803	0.004	0.601	0.002	0.399	0.006
		PM50	0.001	0.790	0.001	0.566	0.001	0.434	0.002
	0.02	None	0.009	0.772	0.012	0.581	0.008	0.419	0.020
		MG5	0.009	0.785	0.012	0.593	0.008	0.407	0.020
		MG10	0.009	0.789	0.012	0.581	0.008	0.419	0.020
		GE5	0.007	0.834	0.008	0.648	0.005	0.352	0.013
		GE10	0.006	0.912	0.007	0.709	0.003	0.291	0.010
		PM25	0.005	0.787	0.006	0.577	0.005	0.423	0.011
		PM50	0.002	0.794	0.003	0.578	0.002	0.422	0.005
	0.03	None	0.013	0.772	0.017	0.590	0.012	0.410	0.030
		MG5	0.013	0.772	0.017	0.561	0.013	0.439	0.030
		MG10	0.014	0.787	0.018	0.588	0.013	0.412	0.031
		GE5	0.011	0.837	0.013	0.651	0.007	0.349	0.020
		GE10	0.009	0.894	0.010	0.682	0.005	0.318	0.015
		PM25	0.007	0.779	0.010	0.572	0.007	0.428	0.017
		PM50	0.003	0.799	0.004	0.576	0.003	0.424	0.008
	0.05	None	0.023	0.770	0.030	0.580	0.021	0.420	0.051
		MG5	0.024	0.800	0.030	0.589	0.021	0.411	0.050
		MG10	0.022	0.767	0.029	0.576	0.021	0.424	0.050
		GE5	0.018	0.841	0.021	0.649	0.011	0.351	0.032
		GE10	0.015	0.899	0.016	0.685	0.007	0.315	0.024
		PM25	0.013	0.777	0.017	0.592	0.011	0.408	0.028
		PM50	0.006	0.786	0.007	0.567	0.005	0.433	0.012
	0.1	None	0.045	0.783	0.057	0.583	0.041	0.417	0.098
		MG5	0.046	0.792	0.058	0.582	0.041	0.418	0.099
		MG10	0.047	0.793	0.059	0.593	0.041	0.407	0.100
		GE5	0.037	0.835	0.044	0.654	0.023	0.346	0.068
		GE10	0.031	0.896	0.034	0.705	0.014	0.295	0.049
		PM25	0.026	0.782	0.033	0.581	0.024	0.419	0.056
		PM50	0.011	0.783	0.014	0.577	0.010	0.423	0.025

Whereas g^2 represents the total genetic variance corresponding to error-free data, σ_{gen}^2 now refers to the empirical total genetic variance.

Table A3 Continued

p	σ_g^2	**Noisiness**	σ_{add}^2	$\sigma_{add}^2/\sigma_{main}^2$	σ_{main}^2	$\sigma_{main}^2/\sigma_{gen}^2$	σ_{epi}^2	$\sigma_{epi}^2/\sigma_{gen}^2$	σ_{gen}^2
0.25	0.01	None	0.000	0.429	0.001	0.103	0.009	0.897	0.010
		MG5	0.000	0.460	0.001	0.100	0.009	0.900	0.010
		MG10	0.000	0.329	0.001	0.142	0.009	0.858	0.010
		GE5	0.000	0.436	0.001	0.135	0.006	0.865	0.007
		GE10	0.000	0.454	0.001	0.166	0.004	0.834	0.005
		PM25	0.000	0.436	0.001	0.113	0.005	0.887	0.006
		PM50	0.000	0.441	0.000	0.126	0.002	0.874	0.002
	0.02	None	0.001	0.408	0.002	0.117	0.018	0.883	0.020
		MG5	0.001	0.414	0.002	0.124	0.017	0.876	0.020
		MG10	0.001	0.391	0.003	0.148	0.017	0.852	0.019
		GE5	0.001	0.473	0.002	0.142	0.012	0.858	0.014
		GE10	0.001	0.556	0.002	0.158	0.009	0.842	0.010
		PM25	0.000	0.395	0.001	0.107	0.010	0.893	0.011
		PM50	0.000	0.361	0.001	0.130	0.005	0.870	0.005
	0.03	None	0.001	0.410	0.004	0.121	0.026	0.879	0.030
		MG5	0.001	0.355	0.003	0.113	0.027	0.887	0.030
		MG10	0.001	0.379	0.003	0.117	0.026	0.883	0.030
		GE5	0.001	0.455	0.003	0.132	0.018	0.868	0.021
		GE10	0.001	0.485	0.003	0.172	0.013	0.828	0.015
		PM25	0.001	0.450	0.002	0.110	0.015	0.890	0.016
		PM50	0.000	0.377	0.001	0.124	0.007	0.876	0.007
	0.05	None	0.002	0.414	0.006	0.118	0.044	0.882	0.050
		MG5	0.002	0.389	0.006	0.120	0.044	0.880	0.050
		MG10	0.003	0.452	0.006	0.115	0.045	0.885	0.051
		GE5	0.002	0.474	0.005	0.147	0.030	0.853	0.035
		GE10	0.002	0.547	0.004	0.160	0.021	0.840	0.025
		PM25	0.001	0.407	0.003	0.124	0.024	0.876	0.028
		PM50	0.001	0.389	0.001	0.118	0.011	0.882	0.013
	0.1	None	0.005	0.407	0.011	0.114	0.088	0.886	0.100
		MG5	0.005	0.403	0.011	0.113	0.089	0.887	0.100
		MG10	0.004	0.367	0.011	0.115	0.088	0.885	0.100
		GE5	0.004	0.454	0.010	0.139	0.060	0.861	0.070
		GE10	0.004	0.508	0.008	0.155	0.044	0.845	0.052
		PM25	0.003	0.400	0.007	0.121	0.049	0.879	0.056
		PM50	0.001	0.366	0.003	0.120	0.022	0.880	0.025

Table A3 Continued

p	σ_g^2	**Noisiness**	σ_{add}^2	$\sigma_{add}^2/\sigma_{main}^2$	σ_{main}^2	$\sigma_{main}^2/\sigma_{gen}^2$	σ_{epi}^2	$\sigma_{epi}^2/\sigma_{gen}^2$	σ_{gen}^2
0.5	0.01	None	0.000	0.374	0.000	0.000	0.010	1.000	0.010
		MG5	0.000	0.778	0.000	0.001	0.010	0.999	0.010
		MG10	0.000	0.758	0.000	0.001	0.011	0.999	0.011
		GE5	0.000	0.795	0.000	0.000	0.007	1.000	0.007
		GE10	0.000	0.591	0.000	0.002	0.005	0.998	0.005
		PM25	0.000	0.363	0.000	0.002	0.006	0.998	0.006
		PM50	0.000	0.898	0.000	0.002	0.002	0.998	0.002
	0.02	None	0.000	0.318	0.000	0.001	0.020	0.999	0.020
		MG5	0.000	0.516	0.000	0.000	0.020	1.000	0.020
		MG10	0.000	0.461	0.000	0.001	0.020	0.999	0.020
		GE5	0.000	0.885	0.000	0.001	0.015	0.999	0.015
		GE10	0.000	0.768	0.000	0.001	0.011	0.999	0.011
		PM25	0.000	0.424	0.000	0.002	0.011	0.998	0.011
		PM50	0.000	0.795	0.000	0.001	0.005	0.999	0.005
	0.03	None	0.000	0.197	0.000	0.000	0.030	1.000	0.030
		MG5	0.000	0.649	0.000	0.000	0.031	1.000	0.031
		MG10	0.000	0.850	0.000	0.000	0.031	1.000	0.031
		GE5	0.000	0.248	0.000	0.000	0.022	1.000	0.022
		GE10	0.000	0.893	0.000	0.001	0.016	0.999	0.016
		PM25	0.000	0.906	0.000	0.000	0.017	1.000	0.017
		PM50	0.000	0.228	0.000	0.001	0.008	0.999	0.008
	0.05	None	0.000	0.561	0.000	0.000	0.050	1.000	0.050
		MG5	0.000	0.552	0.000	0.000	0.050	1.000	0.050
		MG10	0.000	0.974	0.000	0.000	0.050	1.000	0.050
		GE5	0.000	0.851	0.000	0.000	0.037	1.000	0.037
		GE10	0.000	0.852	0.000	0.000	0.027	1.000	0.027
		PM25	0.000	0.899	0.000	0.000	0.027	1.000	0.027
		PM50	0.000	0.675	0.000	0.001	0.012	0.999	0.012
	0.1	None	0.000	0.979	0.000	0.000	0.100	1.000	0.100
		MG5	0.000	0.881	0.000	0.000	0.100	1.000	0.100
		MG10	0.000	0.863	0.000	0.000	0.100	1.000	0.100
		GE5	0.000	0.924	0.000	0.000	0.075	1.000	0.075
		GE10	0.000	0.756	0.000	0.000	0.055	1.000	0.055
		PM25	0.000	0.918	0.000	0.000	0.056	1.000	0.056
		PM50	0.000	0.850	0.000	0.000	0.026	1.000	0.026

Table A4 Empirical variance decomposition for model M27, in the absence of GH.

p	σ_g^2	**Noisiness**	σ_{add}^2	$\sigma_{add}^2/\sigma_{main}^2$	σ_{main}^2	$\sigma_{main}^2/\sigma_{gen}^2$	σ_{epi}^2	$\sigma_{epi}^2/\sigma_{gen}^2$	σ_{gen}^2
0.1	0.01	None	0.003	0.948	0.003	0.324	0.007	0.676	0.010
		MG5	0.003	0.954	0.003	0.298	0.007	0.702	0.010
		MG10	0.003	0.941	0.003	0.314	0.007	0.686	0.010
		GE5	0.002	0.964	0.002	0.363	0.004	0.637	0.006
		GE10	0.002	0.984	0.002	0.407	0.003	0.593	0.005
		PM25	0.002	0.947	0.002	0.318	0.004	0.682	0.006
		PM50	0.001	0.937	0.001	0.345	0.002	0.655	0.003
	0.02	None	0.006	0.953	0.006	0.309	0.013	0.691	0.020
		MG5	0.006	0.954	0.006	0.322	0.014	0.678	0.020
		MG10	0.006	0.939	0.006	0.308	0.014	0.692	0.021
		GE5	0.005	0.969	0.005	0.372	0.009	0.628	0.014
		GE10	0.004	0.981	0.004	0.408	0.006	0.592	0.010
		PM25	0.003	0.963	0.004	0.330	0.007	0.670	0.011
		PM50	0.002	0.971	0.002	0.332	0.003	0.668	0.005
	0.03	None	0.009	0.951	0.009	0.320	0.020	0.680	0.030
		MG5	0.009	0.942	0.010	0.313	0.021	0.687	0.031
		MG10	0.009	0.952	0.010	0.321	0.020	0.679	0.030
		GE5	0.007	0.943	0.007	0.368	0.012	0.632	0.020
		GE10	0.006	0.979	0.006	0.408	0.009	0.592	0.015
		PM25	0.005	0.960	0.005	0.322	0.011	0.678	0.017
		PM50	0.002	0.926	0.002	0.308	0.005	0.692	0.007
	0.05	None	0.015	0.953	0.016	0.312	0.034	0.688	0.050
		MG5	0.016	0.945	0.017	0.324	0.035	0.676	0.051
		MG10	0.015	0.945	0.016	0.311	0.035	0.689	0.050
		GE5	0.012	0.953	0.013	0.372	0.021	0.628	0.034
		GE10	0.010	0.985	0.010	0.419	0.014	0.581	0.025
		PM25	0.008	0.938	0.009	0.313	0.019	0.687	0.028
		PM50	0.004	0.932	0.004	0.319	0.009	0.681	0.013
	0.1	None	0.030	0.943	0.031	0.314	0.068	0.686	0.100
		MG5	0.031	0.946	0.033	0.326	0.068	0.674	0.101
		MG10	0.030	0.942	0.032	0.316	0.070	0.684	0.102
		GE5	0.024	0.953	0.025	0.366	0.043	0.634	0.068
		GE10	0.020	0.979	0.021	0.416	0.029	0.584	0.049
		PM25	0.016	0.946	0.017	0.314	0.038	0.686	0.055
		PM50	0.007	0.941	0.008	0.313	0.017	0.687	0.025

Whereas σ_g^2 represents the total genetic variance corresponding to error-free data, σ_{gen}^2 now refers to the empirical total genetic variance.

Table A4 Continued

p	σ_g^2	**Noisiness**	σ_{add}^2	$\sigma_{add}^2/\sigma_{main}^2$	σ_{main}^2	$\sigma_{main}^2/\sigma_{gen}^2$	σ_{epi}^2	$\sigma_{epi}^2/\sigma_{gen}^2$	σ_{gen}^2
0.25	0.01	None	0.005	0.856	0.006	0.605	0.004	0.395	0.010
		MG5	0.005	0.855	0.006	0.630	0.004	0.370	0.010
		MG10	0.005	0.845	0.006	0.624	0.004	0.376	0.010
		GE5	0.005	0.865	0.006	0.649	0.003	0.351	0.009
		GE10	0.004	0.900	0.005	0.667	0.002	0.333	0.007
		PM25	0.003	0.867	0.004	0.623	0.002	0.377	0.006
		PM50	0.001	0.861	0.001	0.571	0.001	0.429	0.003
	0.02	None	0.010	0.865	0.012	0.617	0.008	0.383	0.020
		MG5	0.011	0.869	0.012	0.625	0.007	0.375	0.020
		MG10	0.010	0.851	0.012	0.613	0.007	0.387	0.019
		GE5	0.010	0.856	0.011	0.637	0.006	0.363	0.018
		GE10	0.009	0.892	0.010	0.658	0.005	0.342	0.015
		PM25	0.006	0.859	0.007	0.624	0.004	0.376	0.011
		PM50	0.003	0.871	0.003	0.628	0.002	0.372	0.005
	0.03	None	0.016	0.852	0.019	0.609	0.012	0.391	0.031
		MG5	0.016	0.864	0.019	0.610	0.012	0.390	0.030
		MG10	0.015	0.861	0.018	0.606	0.012	0.394	0.030
		GE5	0.014	0.870	0.017	0.642	0.009	0.358	0.026
		GE10	0.013	0.885	0.015	0.652	0.008	0.348	0.022
		PM25	0.009	0.864	0.010	0.610	0.007	0.390	0.017
		PM50	0.004	0.853	0.005	0.623	0.003	0.377	0.007
	0.05	None	0.026	0.855	0.031	0.609	0.020	0.391	0.050
		MG5	0.026	0.855	0.030	0.605	0.020	0.395	0.050
		MG10	0.026	0.851	0.030	0.611	0.019	0.389	0.050
		GE5	0.024	0.864	0.028	0.641	0.015	0.359	0.043
		GE10	0.022	0.873	0.025	0.666	0.012	0.334	0.037
		PM25	0.015	0.864	0.017	0.605	0.011	0.395	0.028
		PM50	0.007	0.856	0.008	0.618	0.005	0.382	0.012
	0.1	None	0.052	0.861	0.060	0.609	0.039	0.391	0.099
		MG5	0.053	0.862	0.062	0.609	0.040	0.391	0.102
		MG10	0.052	0.853	0.061	0.611	0.039	0.389	0.100
		GE5	0.046	0.855	0.054	0.632	0.031	0.368	0.085
		GE10	0.044	0.885	0.049	0.656	0.026	0.344	0.075
		PM25	0.029	0.856	0.033	0.599	0.022	0.401	0.056
		PM50	0.013	0.858	0.016	0.612	0.010	0.388	0.025

Table A4 Continued

p	σ_g^2	**Noisiness**	σ_{add}^2	$\sigma_{add}^2/\sigma_{main}^2$	σ_{main}^2	$\sigma_{main}^2/\sigma_{gen}^2$	σ_{epi}^2	$\sigma_{epi}^2/\sigma_{gen}^2$	σ_{gen}^2
0.5	0.01	None	0.006	0.667	0.009	0.844	0.002	0.156	0.010
		MG5	0.006	0.698	0.009	0.845	0.002	0.155	0.010
		MG10	0.006	0.662	0.009	0.854	0.001	0.146	0.010
		GE5	0.005	0.693	0.008	0.874	0.001	0.126	0.009
		GE10	0.005	0.705	0.008	0.875	0.001	0.125	0.009
		PM25	0.003	0.653	0.005	0.851	0.001	0.149	0.006
		PM50	0.001	0.708	0.002	0.857	0.000	0.143	0.002
	0.02	None	0.012	0.675	0.017	0.852	0.003	0.148	0.020
		MG5	0.011	0.655	0.017	0.863	0.003	0.137	0.020
		MG10	0.011	0.660	0.017	0.846	0.003	0.154	0.020
		GE5	0.011	0.697	0.016	0.859	0.003	0.141	0.018
		GE10	0.010	0.702	0.015	0.877	0.002	0.123	0.017
		PM25	0.006	0.667	0.010	0.850	0.002	0.150	0.011
		PM50	0.003	0.677	0.004	0.859	0.001	0.141	0.005
	0.03	None	0.017	0.664	0.026	0.855	0.004	0.145	0.030
		MG5	0.017	0.680	0.026	0.865	0.004	0.135	0.030
		MG10	0.017	0.679	0.025	0.856	0.004	0.144	0.030
		GE5	0.016	0.683	0.024	0.870	0.004	0.130	0.027
		GE10	0.016	0.717	0.022	0.870	0.003	0.130	0.026
		PM25	0.009	0.661	0.014	0.864	0.002	0.136	0.016
		PM50	0.004	0.646	0.007	0.866	0.001	0.134	0.008
	0.05	None	0.028	0.669	0.042	0.854	0.007	0.146	0.049
		MG5	0.029	0.669	0.043	0.858	0.007	0.142	0.050
		MG10	0.028	0.659	0.042	0.851	0.007	0.149	0.050
		GE5	0.027	0.669	0.040	0.871	0.006	0.129	0.046
		GE10	0.026	0.693	0.038	0.876	0.005	0.124	0.043
		PM25	0.016	0.678	0.024	0.858	0.004	0.142	0.028
		PM50	0.007	0.659	0.011	0.856	0.002	0.144	0.013
	0.1	None	0.058	0.672	0.086	0.862	0.014	0.138	0.100
		MG5	0.057	0.669	0.085	0.855	0.014	0.145	0.099
		MG10	0.057	0.667	0.085	0.856	0.014	0.144	0.100
		GE5	0.055	0.682	0.080	0.864	0.013	0.136	0.093
		GE10	0.052	0.701	0.074	0.875	0.011	0.125	0.085
		PM25	0.032	0.663	0.049	0.861	0.008	0.139	0.056
		PM50	0.014	0.665	0.021	0.855	0.004	0.145	0.025

Table A5-i Empirical variance decomposition of the total genetic variance due to (SNP1,SNP2) in model M170, for several GH settings.

p_1	p_2	σ^2_g	σ^2_{add}	$\sigma^2_{add}/\sigma^2_{main}$	σ^2_{main}	$\sigma^2_{main}/\sigma^2_{gen,pair}$	σ^2_{epi}	$\sigma^2_{epi}/\sigma^2_{gen,pair}$	$\sigma^2_{gen,pair}$
0.1	0.1	0.01	0.001	0.789	0.001	0.552	0.001	0.448	0.003
		0.02	0.002	0.789	0.003	0.562	0.002	0.438	0.005
		0.03	0.004	0.793	0.005	0.596	0.003	0.404	0.008
		0.05	0.006	0.770	0.008	0.604	0.005	0.397	0.013
		0.1	0.012	0.782	0.015	0.582	0.012	0.418	0.026
0.25	0.25	0.01	0.000	0.436	0.000	0.133	0.002	0.867	0.003
		0.02	0.000	0.329	0.001	0.112	0.005	0.888	0.005
		0.03	0.000	0.382	0.001	0.117	0.007	0.883	0.008
		0.05	0.000	0.398	0.001	0.119	0.011	0.881	0.012
		0.1	0.001	0.393	0.003	0.109	0.022	0.891	0.025
0.5	0.5	0.01	0.000	0.527	0.000	0.001	0.003	0.999	0.003
		0.02	0.000	0.490	0.000	0.001	0.005	0.999	0.005
		0.03	0.000	0.764	0.000	0.000	0.007	1.000	0.007
		0.05	0.000	0.770	0.000	0.002	0.013	1.000	0.013
		0.1	0.000	0.991	0.000	0.000	0.025	1.000	0.025

Whereas σ^2_g represents the total genetic variance corresponding to error-free data, $\sigma^2_{gen,pair}$ now refers to the empirical total genetic variance due to a single interactive pair.

Table A5-ii Empirical variance decomposition of the total genetic variance due to (SNP1,SNP2) and (SNP3,SNP4) in model M170, for several GH settings.

p_1	p_2	σ_g^2	σ_{add}^2	$\sigma_{add}^2/\sigma_{main}^2$	σ_{main}^2	$\sigma_{main}^2/\sigma_{gen,2pairs}^2$	σ_{epi}^2	$\sigma_{epi}^2/\sigma_{gen,2pairs}^2$	$\sigma_{gen,2pairs}^2$
0.1	0.1	0.01	0.002	0.770	0.003	0.568	0.002	0.432	0.005
		0.02	0.004	0.789	0.006	0.569	0.004	0.431	0.010
		0.03	0.007	0.795	0.009	0.594	0.006	0.407	0.015
		0.05	0.012	0.771	0.015	0.593	0.011	0.408	0.026
		0.1	0.023	0.781	0.029	0.583	0.021	0.417	0.050
0.25	0.25	0.01	0.000	0.405	0.001	0.132	0.004	0.868	0.005
		0.02	0.000	0.386	0.001	0.117	0.009	0.883	0.010
		0.03	0.001	0.360	0.002	0.119	0.013	0.881	0.015
		0.05	0.001	0.416	0.003	0.117	0.022	0.883	0.025
		0.1	0.002	0.396	0.006	0.112	0.044	0.888	0.050
0.5	0.5	0.01	0.000	0.794	0.000	0.002	0.005	0.998	0.005
		0.02	0.000	0.719	0.000	0.001	0.010	0.999	0.010
		0.03	0.000	0.603	0.000	0.000	0.014	1.000	0.014
		0.05	0.000	0.694	0.000	0.001	0.026	0.999	0.026
		0.1	0.000	0.777	0.000	0.000	0.050	1.000	0.050

Whereas σ_g^2 represents the total genetic variance corresponding to error-free data, $\sigma_{gen,2pairs}^2$ now refers to the empirical total genetic variance due the two causal marker pairs.

Table A5-iii Empirical variance decomposition of the total genetic variance due to (SNP1,SNP2) in model M27, for several GH settings.

p_1	p_2	σ^2_g	σ^2_{add}	$\sigma^2_{add}/\sigma^2_{main}$	σ^2_{main}	$\sigma^2_{main}/\sigma^2_{gen,pair}$	σ^2_{epi}	$\sigma^2_{epi}/\sigma^2_{gen,pair}$	$\sigma^2_{gen,pair}$
0.1	0.1	0.01	0.001	0.956	0.001	0.320	0.002	0.680	0.003
		0.02	0.001	0.947	0.002	0.287	0.004	0.713	0.005
		0.03	0.002	0.934	0.002	0.293	0.006	0.707	0.008
		0.05	0.004	0.955	0.004	0.338	0.008	0.662	0.013
		0.1	0.008	0.940	0.008	0.324	0.017	0.676	0.025
0.25	0.25	0.01	0.001	0.853	0.002	0.557	0.001	0.443	0.003
		0.02	0.003	0.859	0.003	0.607	0.002	0.393	0.005
		0.03	0.004	0.856	0.005	0.615	0.003	0.386	0.007
		0.05	0.006	0.857	0.007	0.605	0.005	0.395	0.012
		0.1	0.013	0.845	0.016	0.604	0.010	0.396	0.026
0.5	0.5	0.01	0.001	0.669	0.002	0.849	0.000	0.151	0.003
		0.02	0.003	0.671	0.004	0.868	0.001	0.132	0.005
		0.03	0.004	0.645	0.007	0.869	0.001	0.131	0.008
		0.05	0.007	0.662	0.011	0.863	0.002	0.137	0.013
		0.1	0.014	0.667	0.021	0.855	0.004	0.145	0.025

Whereas σ^2_g represents the total genetic variance corresponding to error-free data, $\sigma^2_{gen,pair}$ now refers to the empirical total genetic variance due to a single interactive pair.

Table A5-iv Empirical variance decomposition of the total genetic variance due to (SNP1,SNP2) and (SNP3,SNP4) in model M27, for several GH settings.

p_1	p_2	σ^2_g	σ^2_{add}	$\sigma^2_{add}/\sigma^2_{main}$	σ^2_{main}	$\sigma^2_{main}/\sigma^2_{gen,2pairs}$	σ^2_{epi}	$\sigma^2_{epi}/\sigma^2_{gen,2pairs}$	$\sigma^2_{gen,2pairs}$
0.1	0.1	0.01	0.002	0.953	0.002	0.314	0.004	0.686	0.005
		0.02	0.003	0.953	0.003	0.307	0.007	0.693	0.010
		0.03	0.004	0.939	0.005	0.307	0.011	0.693	0.015
		0.05	0.008	0.948	0.008	0.322	0.017	0.678	0.025
		0.1	0.015	0.935	0.016	0.323	0.034	0.677	0.050
0.25	0.25	0.01	0.003	0.868	0.003	0.603	0.002	0.398	0.005
		0.02	0.005	0.861	0.006	0.625	0.004	0.375	0.010
		0.03	0.008	0.856	0.009	0.609	0.006	0.391	0.015
		0.05	0.013	0.858	0.015	0.610	0.010	0.391	0.025
		0.1	0.026	0.847	0.031	0.607	0.020	0.393	0.050
0.5	0.5	0.01	0.003	0.675	0.004	0.851	0.001	0.149	0.005
		0.02	0.006	0.666	0.008	0.845	0.002	0.155	0.010
		0.03	0.009	0.656	0.013	0.867	0.002	0.133	0.015
		0.05	0.014	0.659	0.022	0.858	0.004	0.142	0.025
		0.1	0.028	0.665	0.042	0.852	0.007	0.149	0.050

Whereas σ^2_g represents the total genetic variance corresponding to error-free data, $\sigma^2_{gen,2pairs}$ now refers to the empirical total genetic variance due the two causal marker pairs.

Table A6 Theoretically derived proportions of the genetic variance in error-prone or error-free data due to main effects (additive and dominance) or epistasis.

		$\sigma^2_{main}/\sigma^2_{gen}$			$\sigma^2_{epi}/\sigma^2_{gen}$		
Model	***p***	**GE5**	**GE10**	**Other**	**GE5**	**GE10**	**Other**
M27	0.1	0.373	0.420	0.319	0.627	0.580	0.681
	0.25	0.635	0.659	0.609	0.365	0.341	0.391
	0.5	0.865	0.873	0.857	0.135	0.127	0.143
M170	0.1	0.650	0.701	0.581	0.350	0.299	0.419
	0.25	0.139	0.161	0.118	0.861	0.839	0.882
	0.5	0.000	0.000	0.000	1.000	1.000	1.000
		$\sigma^2_{add}/\sigma^2_{main}$			$\sigma^2_{dom}/\sigma^2_{main}$		
Model	***p***	**GE5**	**GE10**	**Other**	**GE5**	**GE10**	**Other**
M27	0.1	0.957	0.979	0.947	0.043	0.021	0.053
	0.25	0.865	0.884	0.857	0.135	0.116	0.143
	0.5	0.680	0.698	0.667	0.320	0.302	0.333
M170	0.1	0.837	0.898	0.780	0.163	0.102	0.220
	0.25	0.447	0.502	0.400	0.553	0.498	0.600
	0.5	0.957	0.979	0.947	0.043	0.021	0.053

Results are presented for 5% and 10% GE scenarios. "Other" scenarios refer to error-free settings, MG5, MG10, PC25, PC50 and GH50.

Table A7 Power of MB-MDR to detect different power definitions from genetic heterogeneity settings (Pair1=SNP1 x SNP2, Pair2=SNP3 x SNP4), and false positives, FP (any pair that is not strictly a functional pair).

				Model M27					Model M170				
	p_1	p_2	σ_g^2	Pair1	Pair2	Pair1 or Pair2	Pair 1 and Pair2	FP	Pair1	Pair2	Pair1 or Pair2	Pair1 and Pair2	FP
No Main Effects Correction	0.1	0.1	0.01	0.014	0.022	0.036	0.000	**0.074**	0.018	0.022	0.040	0.000	**0.110**
			0.02	0.102	0.144	0.234	0.012	**0.130**	0.138	0.108	0.224	0.022	**0.172**
			0.03	0.296	0.306	0.508	0.094	**0.212**	0.294	0.298	0.512	0.080	**0.380**
			0.05	0.650	0.572	0.852	0.370	**0.336**	0.690	0.688	0.906	0.472	**0.688**
			0.1	0.936	0.958	0.994	0.900	**0.742**	0.976	0.980	1.000	0.956	**0.968**
	0.25	0.25	0.01	0.020	0.016	0.036	0.000	**0.116**	0.016	0.020	0.036	0.000	**0.072**
			0.02	0.100	0.094	0.186	0.008	**0.200**	0.096	0.078	0.168	0.006	**0.076**
			0.03	0.226	0.266	0.442	0.050	**0.368**	0.224	0.196	0.378	0.042	**0.066**
			0.05	0.564	0.596	0.830	0.330	**0.628**	0.510	0.546	0.792	0.264	**0.112**
			0.1	0.980	0.964	1.000	0.944	**0.962**	0.960	0.972	1.000	0.932	**0.202**
	0.5	0.5	0.01	0.020	0.044	0.064	0.000	**0.148**	0.016	0.022	0.038	0.000	**0.052**
			0.02	0.070	0.072	0.134	0.008	**0.278**	0.074	0.072	0.138	0.008	**0.062**
			0.03	0.206	0.174	0.332	0.048	**0.520**	0.162	0.172	0.306	0.028	**0.054**
			0.05	0.490	0.488	0.750	0.228	**0.810**	0.494	0.430	0.710	0.214	**0.036**
			0.1	0.938	0.958	0.996	0.900	**1.000**	0.954	0.918	1.000	0.872	**0.080**
Additive Main Effects Correction	0.1	0.1	0.01	0.002	0.002	0.004	0.000	0.024	0.002	0.000	0.002	0.000	0.030
			0.02	0.006	0.006	0.012	0.000	0.036	0.002	0.002	0.004	0.000	0.038
			0.03	0.040	0.024	0.064	0.000	0.036	0.002	0.012	0.014	0.000	0.036
			0.05	0.096	0.106	0.192	0.010	0.014	0.030	0.034	0.062	0.002	**0.060**
			0.1	0.476	0.482	0.718	0.240	**0.058**	0.280	0.286	0.494	0.072	**0.128**
	0.25	0.25	0.01	0.000	0.000	0.000	0.000	0.030	0.012	0.006	0.018	0.000	0.042
			0.02	0.008	0.004	0.012	0.000	0.028	0.078	0.040	0.116	0.002	0.048
			0.03	0.012	0.016	0.028	0.000	0.044	0.152	0.168	0.284	0.036	0.036
			0.05	0.056	0.066	0.118	0.004	0.042	0.438	0.496	0.740	0.194	0.028
			0.1	0.348	0.346	0.586	0.108	**0.074**	0.954	0.962	0.998	0.918	**0.056**
	0.5	0.5	0.01	0.000	0.004	0.004	0.000	**0.054**	0.006	0.006	0.012	0.000	0.030
			0.02	0.008	0.008	0.016	0.000	**0.064**	0.044	0.038	0.076	0.006	0.042
			0.03	0.016	0.014	0.030	0.000	**0.118**	0.108	0.112	0.204	0.016	0.036
			0.05	0.066	0.048	0.110	0.004	**0.180**	0.430	0.336	0.606	0.160	0.026
			0.1	0.312	0.356	0.572	0.096	**0.500**	0.920	0.898	0.994	0.824	**0.054**
Codominant Main Effects	0.1	0.1	0.01	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.024
			0.02	0.000	0.004	0.004	0.000	0.036	0.002	0.000	0.002	0.000	0.026
			0.03	0.016	0.008	0.024	0.000	0.024	0.004	0.008	0.012	0.000	0.032
			0.05	0.036	0.042	0.078	0.000	0.010	0.024	0.030	0.054	0.000	0.028
			0.1	0.260	0.264	0.454	0.070	0.028	0.154	0.162	0.294	0.022	0.028
	0.25	0.25	0.01	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.034
			0.02	0.006	0.006	0.012	0.000	0.018	0.036	0.030	0.066	0.000	0.038
			0.03	0.008	0.012	0.020	0.000	0.030	0.072	0.070	0.136	0.006	0.022
			0.05	0.044	0.032	0.072	0.004	0.026	0.296	0.316	0.540	0.072	0.018
			0.1	0.310	0.280	0.514	0.076	0.028	0.874	0.854	0.976	0.752	0.014
	0.5	0.5	0.01	0.000	0.000	0.000	0.000	0.024	0.002	0.006	0.008	0.000	0.022
			0.02	0.000	0.000	0.000	0.000	0.018	0.024	0.016	0.038	0.002	0.028
			0.03	0.000	0.000	0.000	0.000	0.014	0.078	0.064	0.132	0.010	0.032
			0.05	0.002	0.000	0.002	0.000	0.018	0.324	0.296	0.526	0.094	0.014
			0.1	0.010	0.014	0.024	0.000	0.018	0.894	0.876	0.992	0.778	0.038

Considered analyses methods are MB-MDR without main effects correction, with additive or codominant lower order effects adjustment. False positive percentages higher than 0.05 are highlighted in **bold**.

Table A8 MB-MDR power and false positives under the epistasis model M170

		Power				False Positives		
p	σ_g^2	**No Correction**	**Main Effects Correction**	**Additive**	**Codominant**	**No Correction**	**Additive**	**Codominant**
0.1	0.01	0.326	$MB\text{-}MDR_{adjust}$	0.064	0.036	0.978	0.726	**0.074**
			$MB\text{-}MDR_{1D}$	0.210	0.194		0.744	0.092
			$MB\text{-}MDR_{list}$	0.226	0.208		0.752	0.084
			SR_{perm}	0.252	0.252		0.708	0.078
			$SR_{0.05}$	0.020	0.008		0.624	0.016
			MR_{AIC}	0.002	0.000		0.612	0.006
			SR_{top5}	0.050	0.034		0.638	**0.026**
	0.02	0.878	$MB\text{-}MDR_{adjust}$	0.368	0.202	0.982	0.746	**0.050**
			$MB\text{-}MDR_{1D}$	0.642	0.534		0.748	**0.052**
			$MB\text{-}MDR_{list}$	0.678	0.594		0.776	**0.068**
			SR_{perm}	0.582	0.496		0.728	0.128
			$SR_{0.05}$	0.060	0.014		0.640	0.012
			MR_{AIC}	0.024	0.004		0.620	0.004
			SR_{top5}	0.122	0.050		0.638	0.022
	0.03	0.996	$MB\text{-}MDR_{adjust}$	0.794	0.540	0.996	0.708	**0.060**
			$MB\text{-}MDR_{1D}$	0.856	0.644		0.734	**0.064**
			$MB\text{-}MDR_{list}$	0.874	0.696		0.766	**0.060**
			SR_{perm}	0.632	0.406		0.712	0.120
			$SR_{0.05}$	0.260	0.018		0.606	0.006
			MR_{AIC}	0.216	0.010		0.592	0.004
			SR_{top5}	0.314	0.072		0.630	**0.034**
	0.05	1.000	$MB\text{-}MDR_{adjust}$	0.990	0.940		0.718	**0.050**
			$MB\text{-}MDR_{1D}$	0.992	0.922		0.724	**0.034**
			$MB\text{-}MDR_{list}$	0.994	0.924		0.742	**0.036**
			SR_{perm}	0.874	0.290	1.000	0.686	**0.058**
			$SR_{0.05}$	0.840	0.176		0.618	0.006
			MR_{AIC}	0.842	0.166		0.582	0.002
			SR_{top5}	0.842	0.190		0.630	0.014
	0.1	1.000	$MB\text{-}MDR_{adjust}$	1.000	1.000		0.854	**0.054**
			$MB\text{-}MDR_{1D}$	1.000	1.000		0.854	**0.060**
			$MB\text{-}MDR_{list}$	1.000	1.000		0.852	**0.050**
			SR_{perm}	1.000	0.942	1.000	0.772	**0.054**
			$SR_{0.05}$	1.000	0.930		0.740	0.008
			MR_{AIC}	1.000	0.922		0.710	0.000
			SR_{top5}	1.000	0.938		0.756	0.016

False positive percentage is defined as the proportion of simulation samples for which at least one pair other than the causal pair (SNP1, SNP2) are significant. Power is defined as the proportion of simulated samples of which the causal pair (SNP1, SNP2) is significant. Results are for correction of main effects and for different ways of main effect correction. In **bold** are values within Bradley's liberal criterion of robustness

Table A8 Continued

		Power				False Positives		
p	σ_g^2	**No Correction**	**Main Effects Correction**	**Additive**	**Codominant**	**No Correction**	**Additive**	**Codominant**
0.25	0.01	0.234	$MB\text{-}MDR_{adjust}$	0.264	0.234		0.730	**0.050**
			$MB\text{-}MDR_{1D}$	0.234	0.232		0.740	**0.064**
			$MB\text{-}MDR_{list}$	0.234	0.230		0.736	**0.056**
			SR_{perm}	0.262	0.280	0.988	0.680	**0.058**
			$SR_{0.05}$	0.228	0.214		0.638	0.018
			MR_{AIC}	0.208	0.126		0.614	0.002
			SR_{top5}	0.234	0.226		0.648	0.020
	0.02	0.864	$MB\text{-}MDR_{adjust}$	0.872	0.862		0.724	**0.040**
			$MB\text{-}MDR_{1D}$	0.862	0.856		0.726	**0.050**
			$MB\text{-}MDR_{list}$	0.862	0.860		0.730	**0.048**
			SR_{perm}	0.880	0.874	0.974	0.648	**0.058**
			$SR_{0.05}$	0.858	0.786		0.588	0.004
			MR_{AIC}	0.842	0.728		0.574	0.006
			SR_{top5}	0.864	0.812		0.596	0.010
	0.03	0.996	$MB\text{-}MDR_{adjust}$	0.996	0.996		0.700	**0.054**
			$MB\text{-}MDR_{1D}$	0.996	0.996		0.700	**0.050**
			$MB\text{-}MDR_{list}$	0.996	0.996		0.724	**0.054**
			SR_{perm}	0.996	0.994	0.982	0.662	**0.070**
			$SR_{0.05}$	0.994	0.982		0.590	0.006
			MR_{AIC}	0.986	0.964		0.572	0.000
			SR_{top5}	0.990	0.988		0.602	0.012
	0.05	1.000	$MB\text{-}MDR_{adjust}$	1.000	1.000		0.728	**0.040**
			$MB\text{-}MDR_{1D}$	1.000	1.000		0.732	**0.074**
			$MB\text{-}MDR_{list}$	1.000	1.000		0.746	0.082
			SR_{perm}	1.000	1.000	0.990	0.694	0.096
			$SR_{0.05}$	0.996	0.998		0.608	0.010
			MR_{AIC}	1.000	1.000		0.602	0.006
			SR_{top5}	1.000	1.000		0.630	0.016
	0.1	1.000	$MB\text{-}MDR_{adjust}$	1.000	1.000		0.788	**0.038**
			$MB\text{-}MDR_{1D}$	1.000	1.000		0.806	0.082
			$MB\text{-}MDR_{list}$	1.000	1.000		0.846	0.092
			SR_{perm}	1.000	1.000	0.992	0.820	0.132
			$SR_{0.05}$	1.000	1.000		0.668	0.006
			MR_{AIC}	1.000	1.000		0.630	0.004
			SR_{top5}	1.000	1.000		0.688	0.016

Table A8 Continued

		Power				False Positives		
p	σ_g^2	**No Correction**	**Main Effects Correction**	**Additive**	**Codominant**	**No Correction**	**Additive**	**Codominant**
0.5	0.01	0.196	$MB\text{-}MDR_{adjust}$	0.312	0.436		0.686	**0.044**
			$MB\text{-}MDR_{1D}$	0.196	0.192		0.676	**0.044**
			$MB\text{-}MDR_{list}$	0.194	0.192		0.692	**0.040**
			SR_{perm}	0.214	0.220	0.972	0.616	**0.036**
			$SR_{0.05}$	0.196	0.206		0.588	0.014
			MR_{AIC}	0.166	0.194		0.568	0.006
			SR_{top5}	0.198	0.206		0.598	0.016
	0.02	0.806	$MB\text{-}MDR_{adjust}$	0.896	0.948		0.716	**0.048**
			$MB\text{-}MDR_{1D}$	0.804	0.802		0.728	**0.062**
			$MB\text{-}MDR_{list}$	0.804	0.802		0.730	**0.054**
			SR_{perm}	0.830	0.840	0.984	0.676	**0.044**
			$SR_{0.05}$	0.826	0.818		0.616	0.004
			MR_{AIC}	0.804	0.794		0.604	0.000
			SR_{top5}	0.828	0.826		0.626	0.006
	0.03	0.992	$MB\text{-}MDR_{adjust}$	0.998	1.000		0.716	**0.054**
			$MB\text{-}MDR_{1D}$	0.992	0.992		0.714	**0.040**
			$MB\text{-}MDR_{list}$	0.992	0.992		0.710	**0.036**
			SR_{perm}	0.992	0.992	0.980	0.640	**0.048**
			$SR_{0.05}$	0.990	0.988		0.596	0.010
			MR_{AIC}	0.988	0.986		0.592	0.006
			SR_{top5}	0.990	0.990		0.612	0.010
	0.05	1.000	$MB\text{-}MDR_{adjust}$	1.000	1.000		0.682	**0.054**
			$MB\text{-}MDR_{1D}$	1.000	1.000		0.684	**0.030**
			$MB\text{-}MDR_{list}$	1.000	1.000		0.710	**0.030**
			SR_{perm}	1.000	1.000	0.984	0.640	**0.026**
			$SR_{0.05}$	0.998	0.998		0.584	0.004
			MR_{AIC}	1.000	1.000		0.572	0.000
			SR_{top5}	1.000	1.000		0.586	0.010
	0.1	1.000	$MB\text{-}MDR_{adjust}$	1.000	1.000		0.686	**0.040**
			$MB\text{-}MDR_{1D}$	1.000	1.000		0.680	**0.044**
			$MB\text{-}MDR_{list}$	1.000	1.000		0.684	**0.044**
			SR_{perm}	1.000	1.000	0.986	0.624	**0.062**
			$SR_{0.05}$	1.000	1.000		0.570	0.008
			MR_{AIC}	1.000	1.000		0.534	0.000
			SR_{top5}	1.000	1.000		0.568	0.016

Table A9 MB-MDR power and false positives under the epistasis model M27

		Power				False Positives		
p	σ_g^2	**No Correction**	**Main Effects Correction**	**Additive**	**Codominant**	**No Correction**	**Additive**	**Codominant**
0.1	0.01	0.242	$MB\text{-}MDR_{adjust}$	0.088	0.140		0.672	**0.032**
			$MB\text{-}MDR_{1D}$	0.210	0.210		0.680	**0.058**
			$MB\text{-}MDR_{list}$	0.212	0.216		0.700	**0.050**
			SR_{perm}	0.228	0.244	0.988	0.640	**0.048**
			$SR_{0.05}$	0.070	0.078		0.574	0.008
			MR_{AIC}	0.026	0.046		0.546	0.004
			SR_{top5}	0.084	0.094		0.580	0.010
	0.02	0.826	$MB\text{-}MDR_{adjust}$	0.472	0.616		0.708	**0.028**
			$MB\text{-}MDR_{1D}$	0.774	0.778		0.728	**0.062**
			$MB\text{-}MDR_{list}$	0.732	0.782		0.740	**0.066**
			SR_{perm}	0.680	0.754	0.98	0.676	0.086
			$SR_{0.05}$	0.236	0.274		0.618	0.018
			MR_{AIC}	0.110	0.096		0.592	0.002
			SR_{top5}	0.344	0.414		0.620	**0.030**
	0.03	0.968	$MB\text{-}MDR_{adjust}$	0.834	0.926		0.706	**0.070**
			$MB\text{-}MDR_{1D}$	0.948	0.952		0.712	0.094
			$MB\text{-}MDR_{list}$	0.936	0.952		0.718	0.094
			SR_{perm}	0.824	0.880	0.984	0.676	0.142
			$SR_{0.05}$	0.370	0.388		0.612	0.006
			MR_{AIC}	0.238	0.236		0.590	0.000
			SR_{top5}	0.484	0.538		0.612	0.018
	0.05	1.000	$MB\text{-}MDR_{adjust}$	0.998	1.000		0.732	**0.052**
			$MB\text{-}MDR_{1D}$	0.994	0.994		0.766	0.120
			$MB\text{-}MDR_{list}$	0.996	0.994		0.756	0.126
			SR_{perm}	0.876	0.916	0.998	0.698	0.156
			$SR_{0.05}$	0.636	0.702		0.600	0.012
			MR_{AIC}	0.572	0.638		0.598	0.002
			SR_{top5}	0.692	0.766		0.644	**0.026**
	0.1	1.000	$MB\text{-}MDR_{adjust}$	1.000	1.000		0.672	**0.066**
			$MB\text{-}MDR_{1D}$	1.000	1.000		0.688	**0.064**
			$MB\text{-}MDR_{list}$	1.000	1.000		0.700	**0.062**
			SR_{perm}	1.000	1.000	1.000	0.628	**0.068**
			$SR_{0.05}$	1.000	1.000		0.556	0.010
			MR_{AIC}	1.000	1.000		0.536	0.002
			SR_{top5}	1.000	1.000		0.576	0.020

False positive percentage is defined as the proportion of simulation samples for which at least one pair other than the causal pair (SNP1, SNP2) are significant. Power is defined as the proportion of simulated samples of which the causal pair (SNP1, SNP2) is significant. Results are for correction of main effects and for different ways of main effect correction. In bold are values within Bradley's liberal criterion of robustness

Table A9 Continued

		Power				False Positives		
p	σ_g^2	**No Correction**	**Main Effects Correction**	**Additive**	**Codominant**	**No Correction**	**Additive**	**Codominant**
0.25	0.01	0.266	$MB\text{-}MDR_{adjust}$	0.032	0.042		0.662	**0.048**
			$MB\text{-}MDR_{1D}$	0.176	0.168		0.696	**0.074**
			$MB\text{-}MDR_{list}$	0.152	0.170		0.700	**0.062**
			SR_{perm}	0.160	0.192	0.986	0.636	0.086
			$SR_{0.05}$	0.018	0.016		0.566	0.004
			MR_{AIC}	0.006	0.008		0.542	0.004
			SR_{top5}	0.026	0.018		0.578	0.008
	0.02	0.890	$MB\text{-}MDR_{adjust}$	0.216	0.230		0.702	**0.044**
			$MB\text{-}MDR_{1D}$	0.588	0.558		0.722	0.096
			$MB\text{-}MDR_{list}$	0.516	0.554		0.738	**0.068**
			SR_{perm}	0.440	0.410	0.998	0.666	0.106
			$SR_{0.05}$	0.106	0.094		0.582	0.010
			MR_{AIC}	0.094	0.096		0.570	0.010
			SR_{top5}	0.124	0.104		0.602	0.016
	0.03	0.996	$MB\text{-}MDR_{adjust}$	0.514	0.538		0.734	**0.032**
			$MB\text{-}MDR_{1D}$	0.724	0.700		0.732	0.098
			$MB\text{-}MDR_{list}$	0.698	0.676		0.756	**0.064**
			SR_{perm}	0.588	0.464	0.994	0.710	0.116
			$SR_{0.05}$	0.380	0.336		0.630	0.010
			MR_{AIC}	0.354	0.304		0.602	0.002
			SR_{top5}	0.410	0.330		0.650	0.018
	0.05	1.000	$MB\text{-}MDR_{adjust}$	0.930	0.934		0.732	**0.056**
			$MB\text{-}MDR_{1D}$	0.938	0.926		0.734	**0.064**
			$MB\text{-}MDR_{list}$	0.944	0.930		0.746	**0.042**
			SR_{perm}	0.862	0.838	1.000	0.668	**0.052**
			$SR_{0.05}$	0.840	0.822		0.608	0.004
			MR_{AIC}	0.836	0.796		0.596	0.000
			SR_{top5}	0.854	0.834		0.628	0.022
	0.1	1.000	$MB\text{-}MDR_{adjust}$	1.000	1.000		0.826	**0.062**
			$MB\text{-}MDR_{1D}$	1.000	1.000		0.834	**0.064**
			$MB\text{-}MDR_{list}$	1.000	1.000		0.836	**0.046**
			SR_{perm}	1.000	1.000	1.000	0.716	**0.046**
			$SR_{0.05}$	1.000	1.000		0.660	0.014
			MR_{AIC}	1.000	1.000		0.628	0.002
			SR_{top5}	1.000	1.000		0.678	0.020

Table A9 Continued

		Power				False Positives		
p	σ_g^2	**No Correction**	**Main Effects Correction**	**Additive**	**Codominant**	**No Correction**	**Additive**	**Codominant**
0.5	0.01	0.154	$MB\text{-}MDR_{adjust}$	0.012	0		0.700	**0.048**
			$MB\text{-}MDR_{1D}$	0.084	0.068		0.714	0.100
			$MB\text{-}MDR_{list}$	0.078	0.032		0.734	**0.074**
			SR_{perm}	0.074	0.04	0.980	0.682	0.080
			$SR_{0.05}$	0.006	0		0.582	0.006
			MR_{AIC}	0.004	0		0.570	0.006
			SR_{top5}	0.006	0		0.610	0.016
	0.02	0.712	$MB\text{-}MDR_{adjust}$	0.120	0.006		0.736	**0.050**
			$MB\text{-}MDR_{1D}$	0.332	0.208		0.788	0.182
			$MB\text{-}MDR_{list}$	0.312	0.062	0.994	0.790	0.086
			SR_{perm}	0.248	0.048		0.718	0.096
			$SR_{0.05}$	0.050	0		0.606	0.008
			MR_{AIC}	0.052	0		0.594	0.008
			SR_{top5}	0.062	0		0.620	**0.026**
	0.03	0.970	$MB\text{-}MDR_{adjust}$	0.422	0.014		0.796	**0.042**
			$MB\text{-}MDR_{1D}$	0.556	0.17		0.844	0.144
			$MB\text{-}MDR_{list}$	0.534	0.05		0.848	**0.040**
			SR_{perm}	0.386	0.008	1.000	0.786	**0.058**
			$SR_{0.05}$	0.262	0.002		0.676	0.010
			MR_{AIC}	0.266	0.002		0.658	0.008
			SR_{top5}	0.278	0.002		0.688	0.016
	0.05	1.000	$MB\text{-}MDR_{adjust}$	0.858	0.134		0.898	**0.042**
			$MB\text{-}MDR_{1D}$	0.878	0.132		0.906	**0.054**
			$MB\text{-}MDR_{list}$	0.878	0.112		0.918	**0.032**
			SR_{perm}	0.730	0	1.000	0.876	**0.054**
			$SR_{0.05}$	0.704	0.004		0.834	0.004
			MR_{AIC}	0.688	0.004		0.810	0.002
			SR_{top5}	0.720	0.002		0.852	0.018
	0.1	1.000	$MB\text{-}MDR_{adjust}$	1.000	0.684		1.000	**0.056**
			$MB\text{-}MDR_{1D}$	1.000	0.646		1.000	**0.056**
			$MB\text{-}MDR_{list}$	1.000	0.646		1.000	**0.046**
			SR_{perm}	1.000	0.186	1.000	0.998	**0.050**
			$SR_{0.05}$	1.000	0.172		0.998	0.008
			MR_{AIC}	1.000	0.146		0.998	0.002
			SR_{top5}	1.000	0.178		0.998	0.032

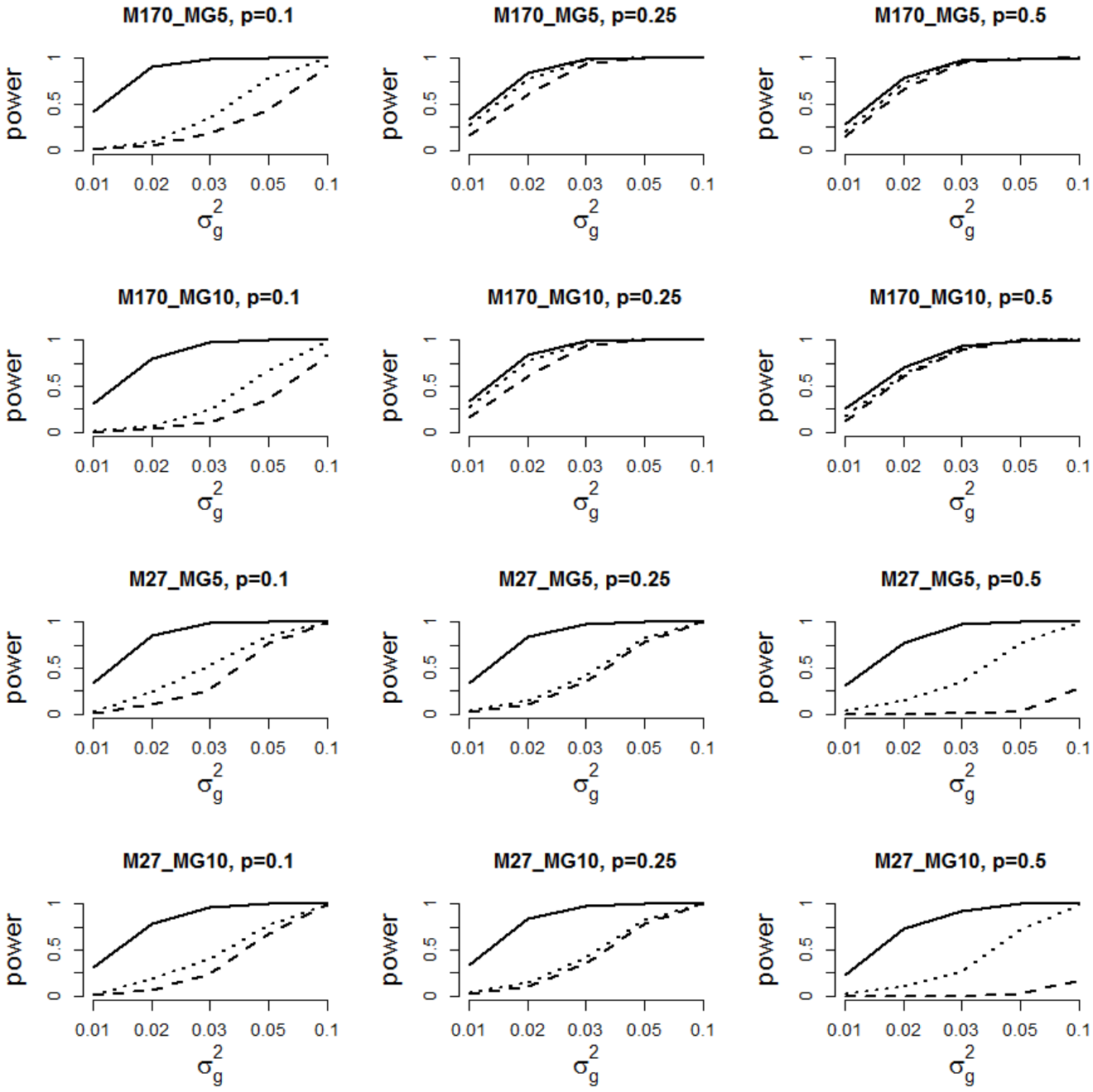

Figure A1-i Empirical power estimates for MB-MDR as the percentage of analyses where the correct interaction (SNP1 x SNP2) is significant at the 5% level, in the presence of 5% or 10% missing genotypes.

Legend no main effects adjustment (—), main effects adjustment via additive coding (…), and co-dominant coding (---).

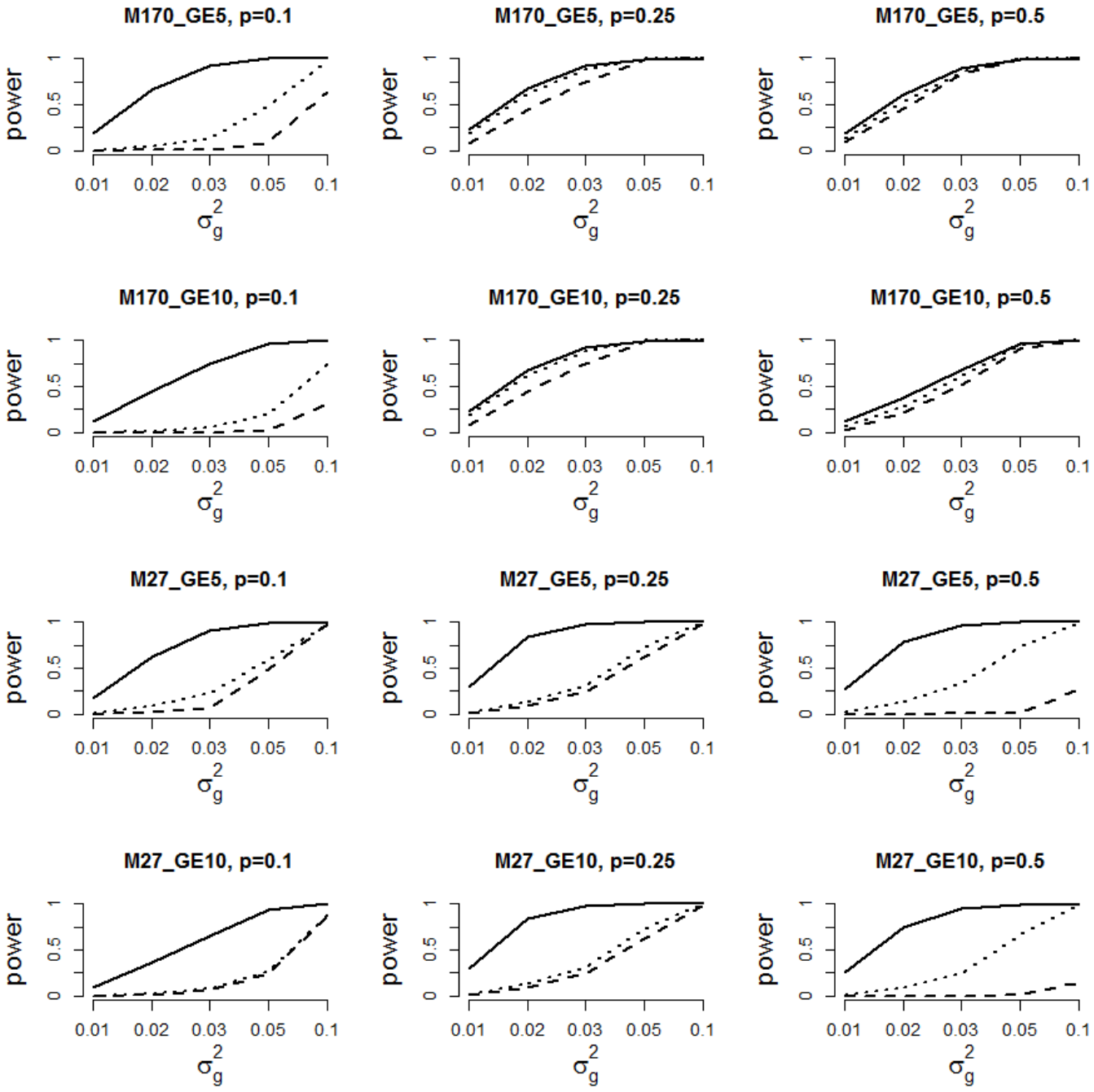

Figure A1-ii Empirical power estimates for MB-MDR as the percentage of analyses where the correct interaction (SNP1 x SNP2) is significant at the 5% level, in the presence of 5% or 10% genotyping errors.

Legend no main effects adjustment (—), main effects adjustment via additive coding (…),and co-dominant coding (---).

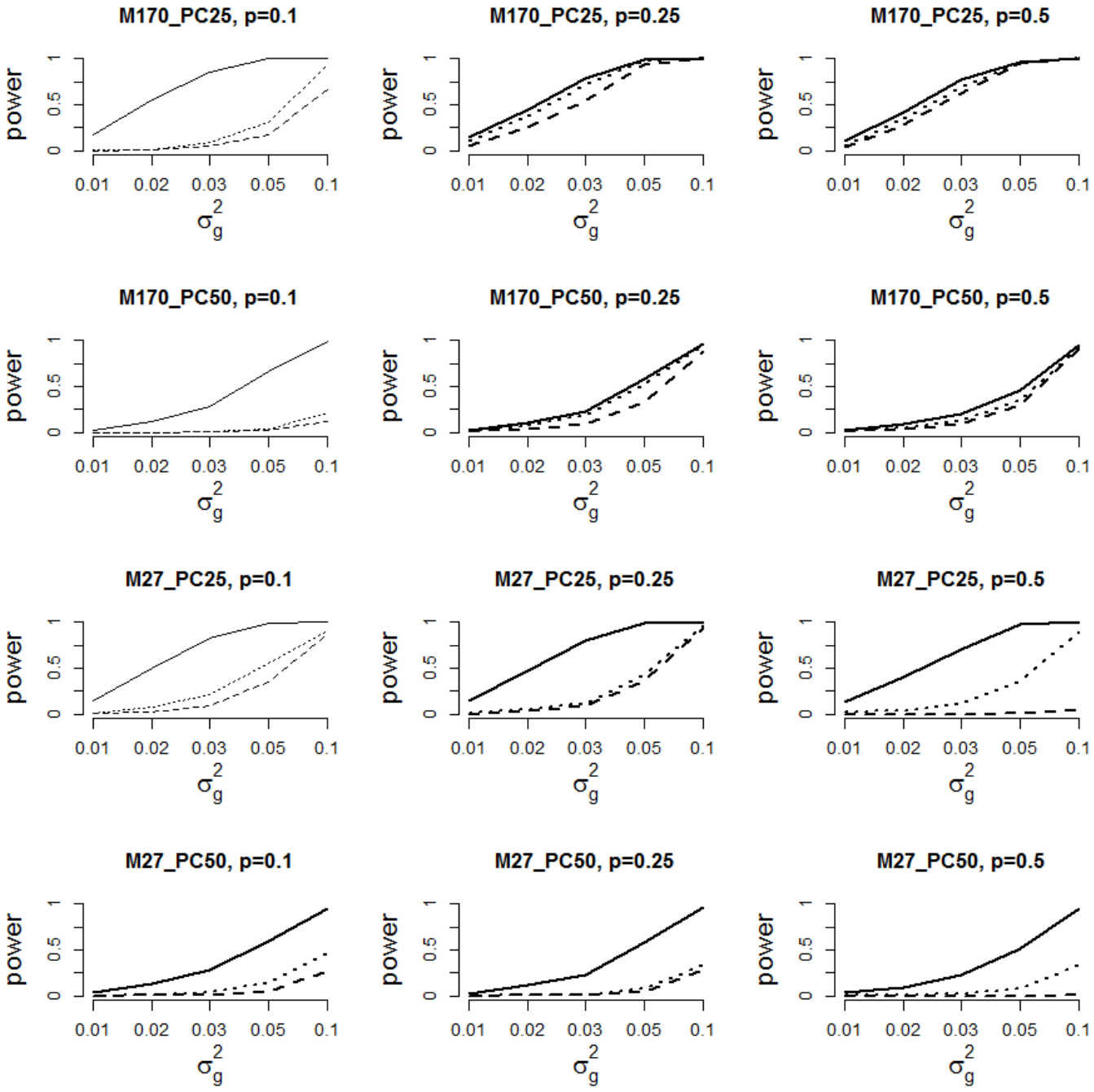

Figure A1-iii Empirical power estimates for MB-MDR as the percentage of analyses where the correct interaction (SNP1 x SNP2) is significant at the 5% level, in the presence of 25% or 50% phenotypic mixture.

Legend no main effects adjustment (—), main effects adjustment via additive coding (…), and co-dominant coding (---).

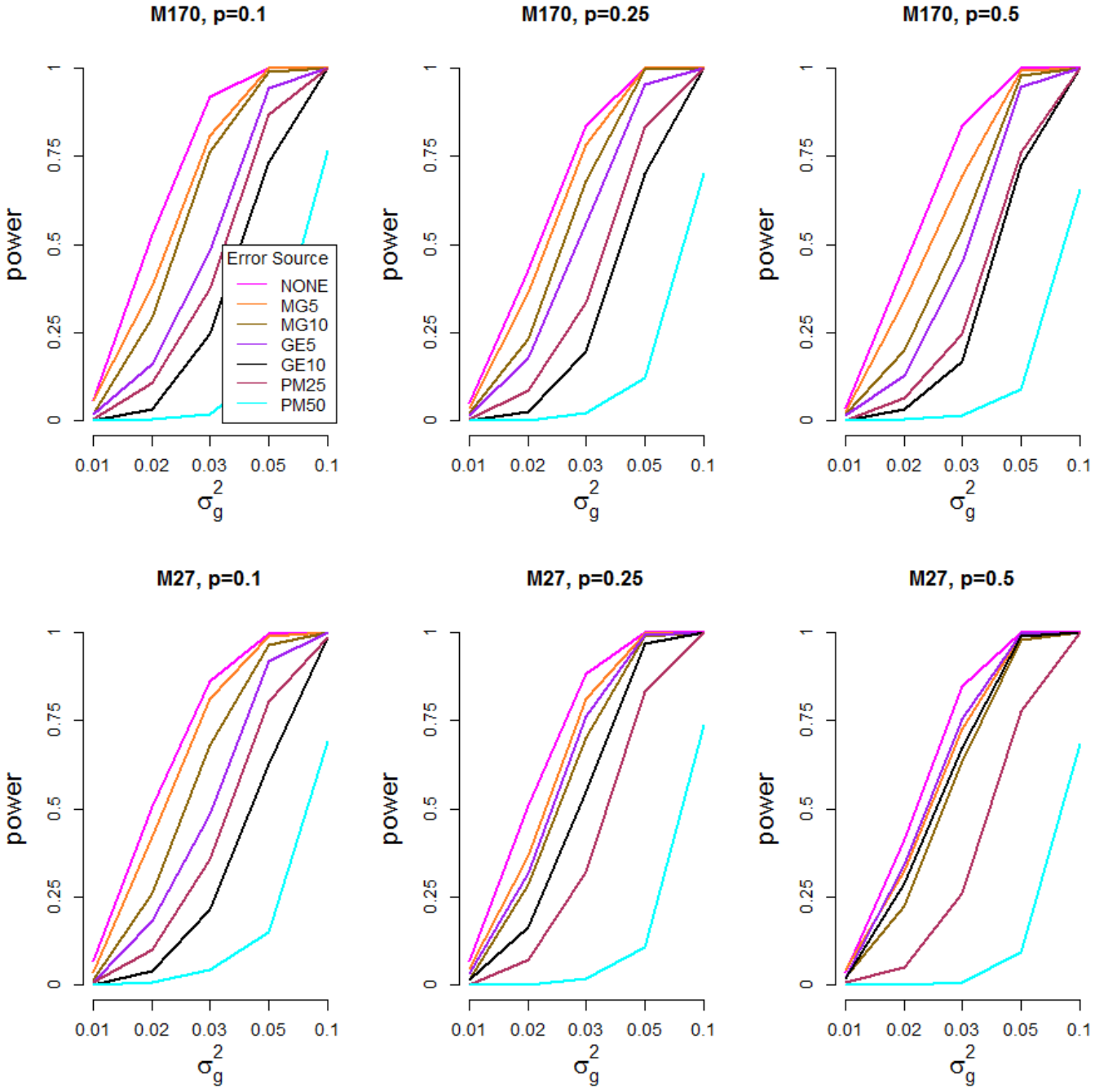

Figure A2 Empirical power estimates of MB-MDR as the percentage of analyses where the correct interaction (SNP1 x SNP2) is significant at the 5% level, for error-free and noise-induced simulation settings involving 250 SNPs.

Legend Results are shown for MB-MDR analysis without main effects adjustment and simulated scenarios other than GH.

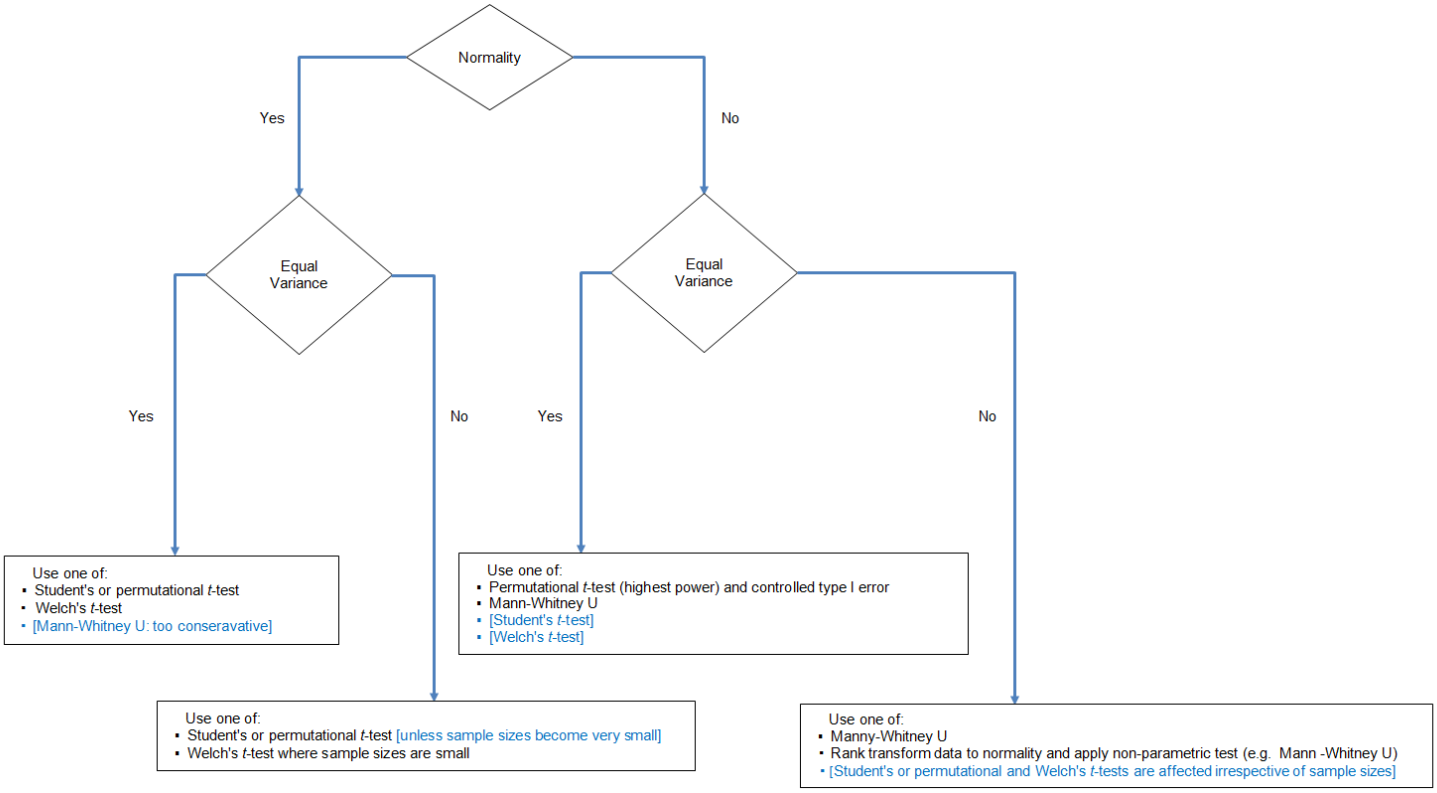

Figure A3 Group comparison test maintaining adequate Type 1 error control, when group sizes are equal.

Legend When several tests are listed, they are listed from most (top) to least (bottom) powerful. The tests in a square box and blue font should be avoided in MB-MDR due to reasons mentioned next to them.

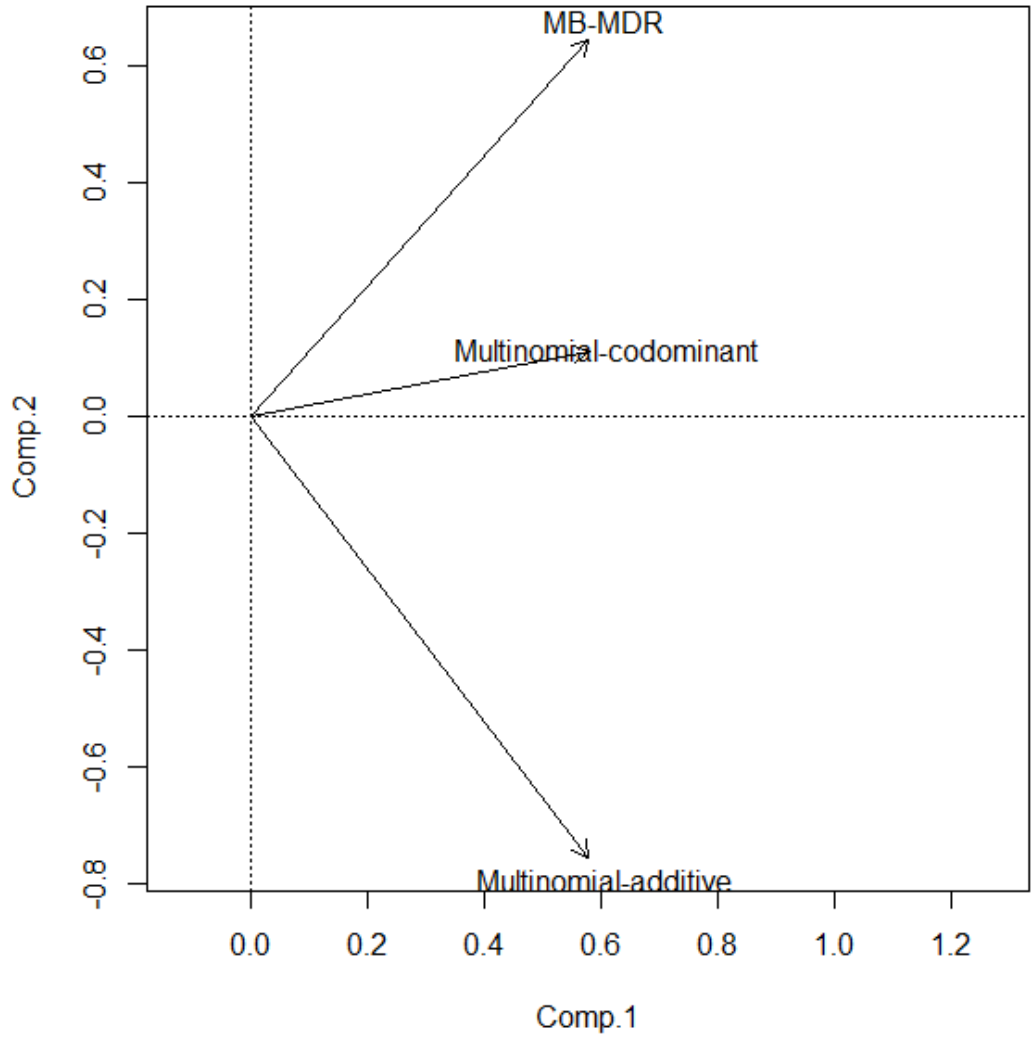

Figure A4 Biplot of the PIAMA data for main effects analysis (MB-MDR and Multinomial).
Legend Data points are suppressed. The cosine of the angle between the lines approximates the correlation between the variables they represent.

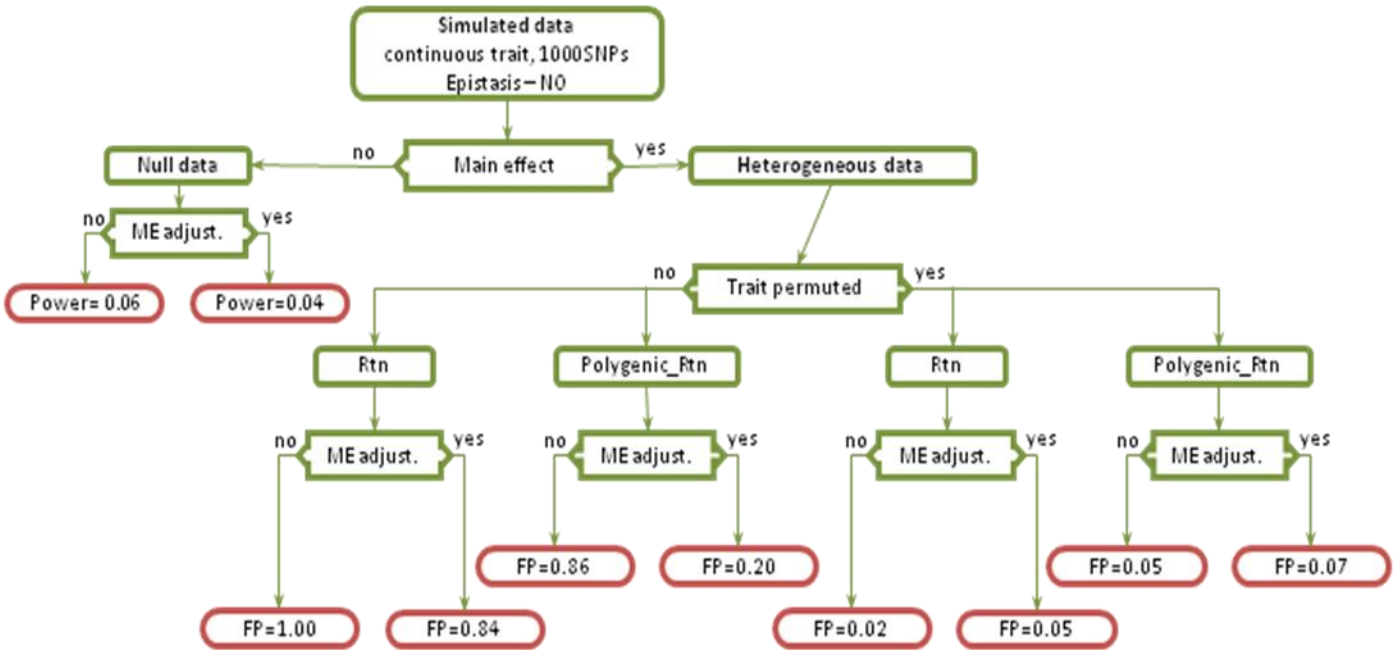

Figure A5 MB-MDR 2-order epistasis screening results.

Legend Rtn– the quantitative trait normalized by rank transformation; Polygenic_Rtn – the residuals derived from the polygenic model applied to the Rtn; ME adjust. – main effect adjustment; FP- False positive rate is estimated as percentage of analyses with identified MB-MDR significant results at alpha level 0.05.

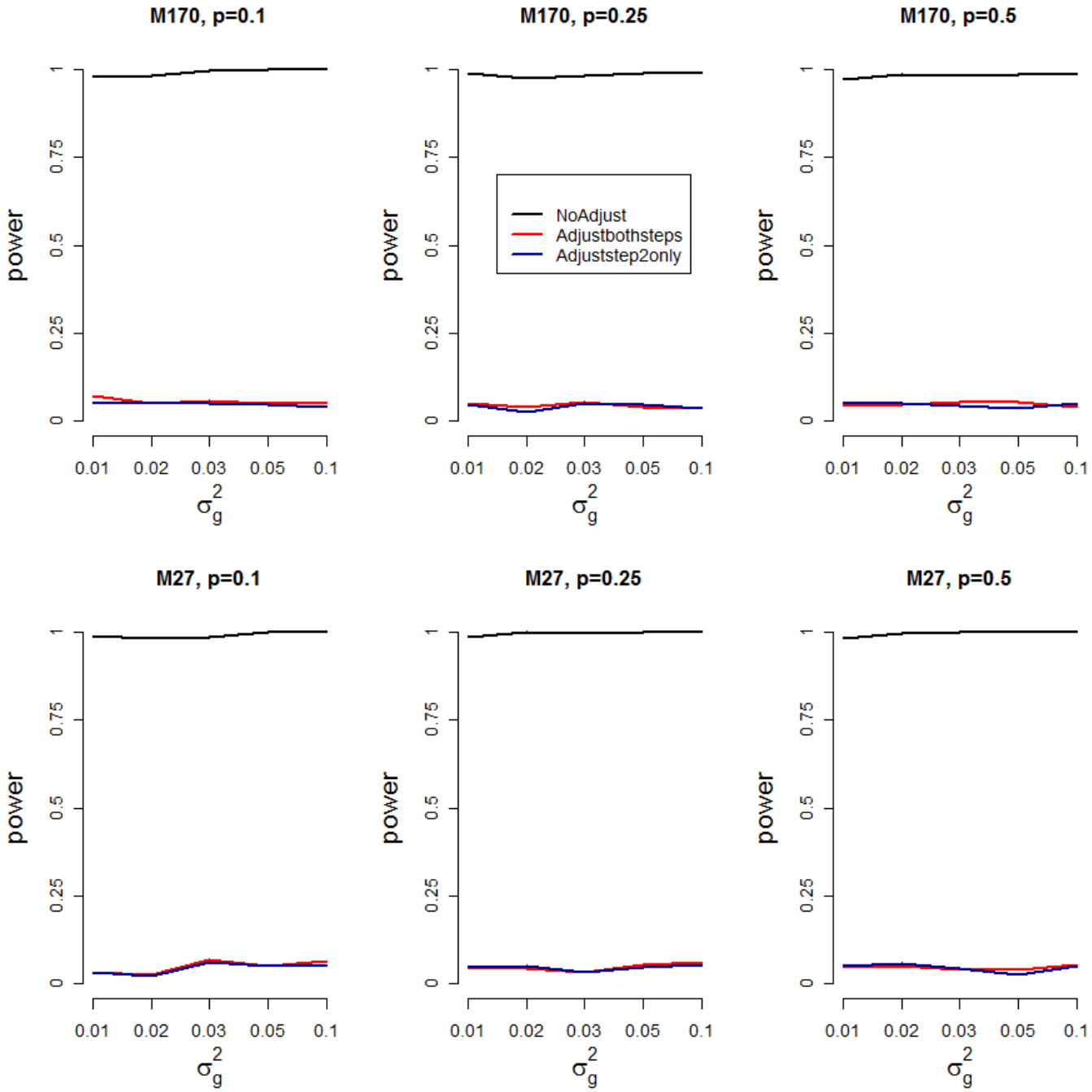

Figure A6 False positive percentage of analyses with identified significant epistasis models other than the correct two functional interacting loci (SNP1 x SNP2).

Legend Profiles are shown for codominant coding when no correction is performed, correction performed at both MB-MDR steps and when correction is only perfomed at step2.

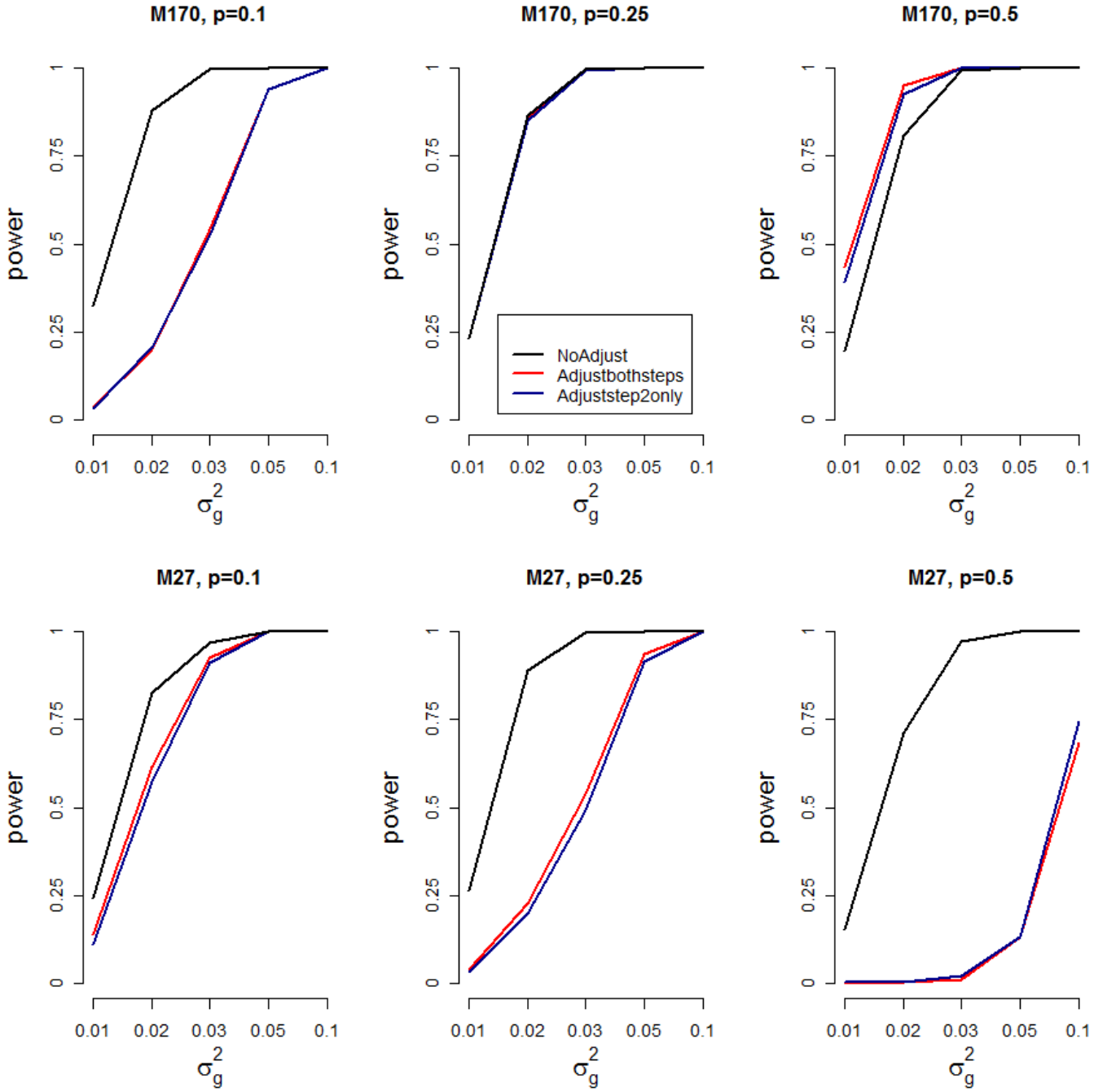

Figure A7 Empirical power estimates for MB-MDR as the percentage of analyses where the correct interaction (SNP1 x SNP2) is significant at the 5% level.

Legend Profiles are shown for codominant coding when no correction is performed, correction performed at both MB-MDR steps and when correction is only perfomed at step2.