# Human Complex Trait Epistasis: The Answer or Still the Question?

Wen-hua Wei (University of Edinburgh and University of Manchester)

Gibran Hemani (University of Queensland and University of Bristol)

Chris Haley (University of Edinburgh)

Correspondence to CSH

e-mail: Chris.Haley@igmm.ed.ac.uk

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## Abstract

Genome wide association studies (GWAS) have become the focus of the statistical analysis of complex traits in humans, successfully shedding light on several aspects of genetic architecture and also biological etiology. Single nucleotide polymorphisms (SNPs) are usually modeled as having linear, cumulative, and independent effects on the phenotype. Though evidently a useful approach, it is often argued that this is not a realistic biological model and that epistasis, the statistical interaction between SNPs, should be included. In this review we discuss the relevance of epistasis in the context of GWAS, recent advances in methodology, evidence of its contribution to complex traits in humans, and potential hazards in the interpretation of statistical interaction terms.

## Introduction

Complex traits or diseases are those that are influenced by multiple environmental and genetic factors. Almost all diseases that have a significant burden on human health are complex at the population scale. Even classically "Mendelian" diseases, such as cystic fibrosis, are at some level complex because numerous genetic effects are involved in modifying the severity of symptoms. Arguably the most important empirical result to emerge from GWAS over the past decade is an indication of what it means for a trait to be "complex", demonstrating that the mutational target size for any particular complex trait across the genome is very large, and that the additive genetic variation is comprised of very many variants, almost all of very small effect.

There exist several methods for estimating the proportion of the phenotypic variance of a trait that is attributed to additive genetic effects (narrow-sense heritability (*h*2)). But unequivocal estimation of the phenotypic variation attributable to all genetic effects (including dominance and epistasis, broad-sense heritability (*H*2)) is not possible for human traits.1 GWAS are typically performed on traits that have been shown to have a non-zero *h*2, on the assumption that this additive genetic variation in the trait can be dissected into additive effects across the genome. Detecting non-additive genetic effects imposes an assumption that, beyond the additive component of genetic variation, for which there is empirical evidence, there exist more complex components whose existence cannot be verified empirically due to technical limitations. The measurable gap between the variance explained by all known additive effects detected by GWAS and *h*2 is termed the "missing heritability". The proportion of the genetic variation that might exist beyond additive variance might be termed the "unknown heritability".

In essence, there is no strict hypothesis-driven precedent for searching for epistasis as there is for additive effects, and yet the question of epistasis in complex traits is often at the forefront of debate. In this review we will survey emerging methodology for the detection of epistasis, summarize current examples of epistasis impacting human complex traits from the literature, discuss the contexts in which epistasis may or may not be important, and suggest guidelines on what constitutes credible statistical support from empirical studies and how this should be reported.

## Methods for detecting epistasis

Despite the severe challenges in robust detection of epistasis for human complex traits (BOX1), the past five years have seen rapid development of methods for studying epistasis in human complex traits/diseases2–7. Methods ranging from conventional regression-based methods to nature-inspired algorithms are continually being developed (Figure 1). Most methods use SNP-based tests for pairwise or high order interactions in GWAS data via either an exhaustive search of all SNP combinations or testing of a reduced, preselected set. In addition, methods have been developed to assess interactions between groups of SNPs (e.g. genes) or functional modules (e.g. pathway or network). As a broad generalization, approaches that search all pairs of SNPs for evidence of all types of epistasis can be termed “hypothesis free” whereas those which restrict themselves to searching subsets of SNPs and/or types of epistasis may be termed “hypothesis driven”. Because of the size of the parameter space to search and the number of analyses performed, hypothesis free approaches pose a major challenge and one of the key recent achievements in recent years is that computational barriers have been bypassed and an exhaustive search for pairwise interactions has become a more routine exercise8–12. Here we provide an overview of the developments since Cordell’s review13 with a focus on hypothesis-free, genome-wide methods.

### Regression based methods

Regression-based methods are commonly used to assess SNP interactions in either diseases or quantitative traits13,14. In GWAS where billions of pairwise SNP combinations need to be assessed, the primary goal is to identify interacting SNPs from the huge search space. One can use SNP genotype models to test interactions directly by comparing the saturated model including interactions (LS) against the reduced model without (LR), using four degree-of-freedom (df) and thus save computing time in estimating genetic parameters. When concerning only additive effects, the genotype model is reduced to an allelic (i.e. the minor alleles of each SNP) model where the interaction is tested in the same way but using only one df15. Previously an exhaustive search for pairwise interactions at the genome-wide level was considered computationally prohibitive13. Various approaches have been taken to reduce the computational barrier. First, advantage has been taken of modern computing infrastructure and technologies including clusters of computers equipped with multiple CPU cores and/or graphic processing units (GPU)9,11,12,16, parallelization8,10,17 and bitwise computing where SNP genotype data are stored in bitwise data structures to achieve great memory efficiency and computing speed8,18,19. Second, approximate interaction tests have been applied that can be quickly computed and do not miss any important epistatic SNP pairs19. For example F ratio and Kirkwood Superposition Approximation approximate the (LS vs. LR) tests under the assumption of Hardy-Weinberg Equilibrium (HWE) for quantitative20 and disease traits19 respectively and can be quickly computed from contingency tables based on SNP genotypes. For convenience, we list some recent applications based on regression and other approaches that can perform fast genome-wide screening of epistasis in GWAS (TABLE 1). Considering the various strengths and weaknesses in these applications, we recommend an extra step of re-examining the screening results of more significant tests in the full regression models (e.g. conditional tests) to avoid false positives or redundant signals10,20,21.

An exhaustive genome-wide search is now computationally tractable but still suffers from low power in detection of epistasis, especially when applying a genome-wide threshold adjusted for billions of pair-wise tests13,22,23. Big sample sizes are generally required for success24. Focusing on interactions involving SNPs with important marginal effects may be a practical compromise25,26 in light of limited samples available in most individual GWAS cohorts, because a much less stringent threshold can be applied owing to much reduced multiple tests10,20,27–29. Taking 500 000 SNPs for example, the Bonferroni corrected threshold on the -log10 scale is 12.4 for an exhaustive search in contrast to 7.0 for a hypothesis-driven search focusing on SNPs with marginal effects, i.e. the approximate 2x difference in threshold is equivalent to a doubled sample size in the focused search. Furthermore, using high density SNPs could potentially make both exhaustive and focused interaction search more fruitful as power is function of interaction effects and LD between the SNP and causal variant at both loci21,24.

### LD and haplotype based methods**.**

In disease traits, methods based on the difference of inter-locus associations between cases and controls may be more powerful than the logistic regression mainly because such difference can be tested using 1 df χ2 statistic in contrast to 4 df in regression13,15,30. Intuitively, if a haplotype of two SNPs tagging a causal variant of a disease has a higher frequency in cases than in controls, i.e. the inter-locus associations differ between cases and controls, it can generate apparent epistasis illustratable in a genotype-phenotype map27 or a contingency table where each joint genotype is a combination of two of the four possible haplotypes31,32. Note that in this case each SNP will be in LD with the causal variant, but the two SNPs need not be in LD with each other. Unfortunately haplotypes are not directly observed in GWAS and require linkage phase of SNP genotypes to be estimated in advance.

Assuming HWE and linkage phase known, an LD-based statistic was first used to measure inter-locus associations and indeed had a power gain32, this approach was recently implemented in SIXPAC to perform fast pairwise genome scans18 but with several issues to be addressed to become more applicable for GWAS: HWE does not always hold15; phasing SNP genotype data is computational expensive; possible intra-locus interactions21,33 are ignored. Correlation-based measures of LD do not require HWE or phasing34 and have been increasingly used in studying epistasis in GWAS15,16,35. For example, for each pair of SNPs one can compute their Pearson’s correlations in cases and controls separately and derive a Z-score statistic based on the difference to test interactions16,34. Unfortunately, the Z-score statistic is still subject to inflated false positives when the two SNPs are in LD and/or with marginal effects15. This may be cured in the logistic regression model35. Therefore a two-step solution using the Z-score statistic for screening and logistic regression models for testing16 can control false positives without losing the power advantage in the LD-based methods.

Haplotype-based methods face an additional issue: uncertainty in estimating haplotype frequencies especially when the HWE assumption does not hold15,36. An improved haplotype-based statistic that adopts correct variance calculation and incorporates a weight average of the joint effects of two SNPs is as powerful as the Z-score statistic34 and can control false positives when only one SNP has marginal effects, but still generates an inflated false positive rate for the detection of epistasis, particularly if both SNPs have marginal effects and are in LD15. Arguably such inflation could be viewed as strengthen of the haplotype-based methods in terms of identifying ‘co-associated’ marginal loci without interactions35,37 if they are missed in conventional GWAS. The two-step solution16 may be an effective fix of the inflation issue here.

### Bayesian methods

Bayes’ theorem offers a great flexibility to model and stochastically search epistasis without enumerating all SNP combinations13. In BEAM, an early Bayesian tool for GWAS38, detection of interacting SNPs is equivalent to partitioning of independent SNPs (i.e. no LD) into predefined groups according to their posterior probabilities without explicitly testing interactions7,13. Improved BEAM methods use new variables to account for LD among SNPs and thus allow a full analysis of GWAS data7,39, but may benefit more from additional tests for interactions among SNPs partitioned in the target group6. Hybrid Bayesian methods appear to be able to improve detection of epistasis in GWAS40–42, e.g. combining the strengths of Bayesian framework and generalized linear model allows fast and stable tests of SNP or haplotype interactions while considering covariates, marginal effects and gene-environment interaction simultaneously41,43. Besides, the Bayesian model averaging approach may increase power of detection by averaging evidence from multiple plausible models given unknown actual interaction types44.

### Data filtering methods

An alternative hypothesis driven approach is to select a subset for interaction tests based on either existing biological knowledge (e.g. databases of pathways and protein-protein interactions5,24,45), or statistical features (e.g. marginal effects24 and SNP genotype frequencies46,47), or fast algorithms18,48–51. Methods based on variance heterogeneity among SNP genotypes can effectively select potentially interacting SNPs for quantitative traits but could miss SNPs that are interacting but have limited variance heterogeneity52,53. Besides the apparent speed advantage, filtering based methods can be better than exhaustive search in power because of much reduced multiple tests as well as functional interpretation when considering only functional SNPs. However, caution is recommended when applying filtering because of potential biases (either upwards or downwards) caused by limitations in the algorithms and existing knowledge that may be subject to publication bias4 and specific contexts54,55. Furthermore, it is debatable what threshold is appropriate after filtering as it might alter the null distributions of test statistics 56.

### Machine learning, data mining and other algorithms

Many attempts have been made to adopt/improve algorithms from other disciplines to address the large P small N problem in detecting epistasis3,5,6,13, particularly high-order (multiple locus) interactions where regression-based methods3 may suffer from increasingly computational complexity and data sparsity50,57. These algorithms often employ certain classifiers for data reduction and/or feature selection to reduce both the computational and statistical burden of an exhaustive search6,58,59, but previously were not scaled up for GWAS or explicitly testing interactions and could miss interactions without marginal effects13,58,59. While most existing algorithms (e.g. Multifactor Dimensionality Reduction, tree-based, entropy-based) being scaled up by modern computing technologies60–64, their classifiers are also improved to be applicable for complex quantitative traits60,65–68, allow using risk scores aggregating multiple interactions in classification72, and account for interactions without marginal effects69,70.

Improved computing efficiency also allows an easy fix of the stability issue observed in filtering methods derived from the RelifF algorithms71 by aggregating multiple runs72 and facilitates the development of combining complementary algorithms. For example, using tree-based methods for screening and Multifactor Dimensionality Reduction for interaction testing can improve the overall performance73–75; Reconstructability Analysis method uses entropy-based methods to construct and interpret interaction structures and graph theory heuristics to traverse64. New algorithms such as Ant Colony Optimization mimicking how ant colonies find the shortest route to foods76 are being adopted for epistasis studies but need to be clear how interactions are tested. Nevertheless, detection of high-order interactions appears very challenging to machine learning and data mining methods too considering that interactions are not explicitly tested in many cases. When interactions are explicitly tested, e.g. using Reconstructability Analysis, detection of three-way interactions requires multilayer hypothesis tests and thus is as difficult as regression methods4,77, in addition to issues of excessive computing demand, exponentially increased multiple tests and insufficient sample sizes13. Despite some progress, it seems likely that new approaches will be needed for robust genome-wide detection of high-order interactions.

### Group and module based methods

Testing interactions based on SNPs grouped in genes or functional modules can dramatically reduce the multiple test burden (e.g. only ~2 x 108 pairwise tests required for 20,000 genes) and thus increase power of detection23,78, including high-order interactions79. In addition, proper grouping of SNPs may collectively capture casual variants that are not well tagged by individual SNPs80 . However, this may be complicated by a number of factors (e.g. group definitions, correlations among SNPs and SNP pairs)36. A common practice is to use genes to group SNPs and derive gene-based variables factoring in SNP correlations for interaction tests via regression81 or analogous LD-based15 (i.e. testing the difference of correlation of a pair of genes between cases and controls) approaches82,83. Alternatively, one can first compute all pairwise interactions between SNPs in each group and derive gene-based interaction P values by integrating the pairwise interaction P values78.

These gene-based studies suggest it is important to incorporate external LD information (e.g. via imputation) to capture unobserved causal variants and use a composite value from a set of signals (instead of the single best signal, for example) to detect multiple interactions between a pair of genes. Gene-based methods implicitly assume no intragenic interactions which may not necessarily hold21,33. One can also use pre-computed pairwise SNP interactions to build statistical interaction networks and aggregate multiple gene-gene interactions and marginal effects using network analysis algorithms such as SNPrank84 to identify functional important pathways3. However, the issues associated with the gene-based approaches (e.g. intragenic interactions and correlated SNP pairs) also need to be addressed.

### Multi-trait and multi-level integration

Collectively analyzing multiple complementary traits may help detection pleiotropic epistasis for better biological interpretation but this domain remains fundamentally unexplored due to its potential complexity85,86. Pleiotropic epistasis could be identified in human complex traits by simply looking for SNP-SNP interactions shared across related traits21 or using a gene-based method with a latent variable representing multiple traits87. On the other hand, emerging evidence of epistasis from eQTL and other -omics studies can help understand the underlying molecular mechanisms88,89. However, integration of statistical and functional interactions is not straightforward90 and may require system biology approaches91.

### Comparing alternative analytical approaches

So far we have briefly discussed only the major method developments in the past five years. Obviously, it is very difficult to recommend the ‘best’ applications without careful comparisons because of huge variation among them, including interaction definitions and null distributions56. Several attempts have been made to evaluate different methods previously58,59,92–96, suggesting community-wide efforts97 may be essential to fulfill the task. Furthermore, consensus significance thresholds and standards for replication and reporting have become fundamentally important to improve the overall quality of future epistasis studies including result sharing.

## Overview of empirical evidence for epistasis

The literature is replete with reports of epistasis influencing human traits, but often the evidence supporting such claims is difficult to evaluate. A range of different methods and experimental designs have been used, ranging from filtering approaches such as hypothesis-driven candidate gene testing, to hypothesis-free exhaustive searches. And indeed epistasis has been reported for a wide spectrum of complex traits and diseases. Here we provide a summary of some of these findings.

### Hypothesis-free studies

The Welcome Trust Case Control Consortium (WTCCC) data has been fruitful in identifying **marginal** additive effects of modest size, and indeed exhaustive two-locus searches have been applied also. Wan *et al*.19 used BOOST software to perform a search for pairwise interactions in each of the seven traits, and reported thousands of significant interactions in total. The vast majority of interactions were between SNPs within the MHC region affecting type 1 diabetes or rheumatoid arthritis, which may be attributable to haplotype effects and had a multiplicative effect, which may suggest a scale effect. However, some of the multiplicative interactions were between SNPs that have not previously been identified by GWAS, and this lends empirical support to the idea that searching for epistasis may confer increased statistical power to detect marginal effects. Using a different statistical model and more stringent controls for population stratification, Lippert *et al*.98 also performed exhaustive scans for the seven diseases in WTCCC. Their results largely echoed those presented in Wan *et al*., but again there was no attempt at replication to verify these statistical claims.

Prabhu and Pe’er used their computationally efficient software, SIXPAC, to search for epistasis influencing bipolar disorder, and identified a pair of interacting SNPs that had not previously been shown to have an effect from GWAS. They attempted to replicate these results, however although the interacting regions showed some evidence for replication, the actual discovery SNPs did not.

The trend that emerges is that there are hints of epistasis being uncovered through exhaustive searches for complex traits, but as of yet there is no evidence that this approach detects epistatic interactions that are easily interpreted and statistically replicated. Resonating with this conclusion is a study by Lucas *et al*.,99 which restricted the search for epistatic effects influencing risk of myocardial infarction to either SNPs that had weak marginal effects or to SNPs that had known marginal effects for a number of related traits. Though the sample size was reasonably large and the search space was drastically reduced, no statistically significant epistatic signals were uncovered. Similar conclusions have been drawn for other complex traits too, including type 2 diabetes100, body mass index (BMI)101 and serum uric acid levels102. Is this lack of findings the result of underpowered studies, or do they demonstrate that epistasis simply doesn’t comprise any substantial amount of variance in complex traits? Some evidence is now emerging that both conclusions are likely to be true.

In an attempt to maximize detection power, one method is to choose traits for which genetic effects are expected to be large, such as is the case in gene expression103. Using the Brisbane Systems Genetics Study104 (BSGS) data of 846 individuals with gene expression levels measured in whole blood, it was demonstrated that multiple instances of epistatic effects could be detected using an exhaustive pairwise search method, with significant replication in two independent samples105. An important conclusion from the study was that even after correcting for power discrepancy substantially more genetic variance was attributable to additive effects than to non-additive effects, and this is consistent with the observation that identifying robust epistatic signals in higher level complex traits is difficult.

### Hypothesis-driven studies

An alternative approach to performing exhaustive searches is to overcome the problem of having a very stringent threshold by restricting the search to a few candidate loci. Using this strategy, there are some examples of epistasis with strong statistical support, and many examples with weak statistical support in the literature.

The sheer volume of reports of epistasis is exemplified by an important study by Combarros *et al*.106, where they collated data from over 100 publications that reported epistasis of some form influencing Alzheimer’s disease (and related traits such as age of onset). These reports comprised multiple experimental designs, sample sizes, and statistical methods, but they demonstrate that, using a standardized statistical test, 27 had interaction terms at the nominal significance level of *p < 0.05* (mostly involving *APOE4*). Though, ostensibly, this is more than what is expected by chance, Combarros *et al*. pointed out that there are many limitations to how these studies were conducted, notably, most of the studies did not adjust for relevant covariates, nor did they show evidence of replication.

The Epistasis Project was created for this reason, to provide a regularised framework for replication of claims of epistasis in a large, well-controlled study. To date, a few reports of epistasis from candidate gene studies have been discovered107–109 or shown to replicate110 in the Epistasis Project cohort, but perhaps the main conclusion from this work is that one must be cautious in reporting or interpreting epistasis because it appears that in the absence of solid replication we cannot refute the argument that the results are consistent with the majority being false positives.

Beyond the Epistasis Project other statistically robust examples of epistasis have also been shown. For example, Rhinn *et al.*111 designed a study to identify differential gene expression caused by *APOE4* and independent of *APOE4* in conferring a risk for late onset Alzheimer’s disease. In doing so, they demonstrated two genetic interactions where SNPs regulating *FYN* and *RNF219* each decreased the risk of Alzheimer’s in *APOE4* non-carriers, but not in *APOE4* carriers. This finding was also statistically replicated in independent samples. The strategy of restricting the search to genetic effects that control **endophenotypes** that are involved in the trait of interest is an attractive idea, because it is expected that genetic effects influencing endophenotypes will be larger than those influencing higher-order traits, perhaps due to their less **polygenic architecture**.

Multiple sclerosis (MS) is another complex trait in which epistasis has been demonstrated to have an impact. Because the *HLA-DR2* haplotype in the MHC region showed complete linkage disequilibrium over a long distance in multiple ethnic populations it was hypothesised that selection was maintaining the co-segregation of two alleles due to epistasis. *In vivo* studies in humanised mice confirmed that separation of the two alleles led to an MS-like phenotype112, and subsequent analysis in human populations showed increased incidence of MS amongst individuals exhibiting recombination between the interacting loci.113

An alternative strategy for narrowing the search to overcome large significance thresholds is to only test for epistasis amongst SNPs that have known marginal effects. Though not routine, many GWA studies report that they performed follow-up analysis of epistasis amongst their hits, but though the number of positive findings remains very low, some successes have been reported. Strange *et al*.26 looked for epistasis amongst significant marginal effects from a GWA study for psoriasis, and demonstrated that the risk alleles at the *HLA-C* and *ERAP1* loci only conferred effects if they were both present. A similar pattern of epistasis was uncovered using the same strategy by Evans *et al*.25 in a GWA study for ankylosing spondylitis, this time between *ERAP1* and a large additive effect at *HLA-B27*. In both cases, the same patterns replicated in independent samples, and these are perhaps the first statistically robust examples of epistasis influencing human complex traits.

One criticism of these examples of epistasis is that they are evident on the **observed scale** of the disease trait, but because the interaction is between SNPs with large effects, it could be the case that on the **liability scale** of disease the contribution to risk is purely additive. This is indeed a philosophical quandary when dealing with **binary phenotypes**, because on the observed scale one is implicitly using an epistatic threshold model, in the sense that the contribution of a genetic effect to an individual’s risk of becoming affected by a disease depends on the sum of risk alleles elsewhere in the genome.114 This non-linearity is particularly elevated when prevalence in the population is low, as is the case for most complex diseases.

Given the success of identifying epistasis through endophenotypes, and by restricting the search to SNPs with known marginal effects, it is intuitive that combining these strategies should be fruitful, consistent with the identification and replication of instances of epistasis influencing gene expression by Hemani *et al*. However, power is still an important factor using this study design. For example, Becker *et al.*89 also searched for epistasis influencing gene expression but using a much smaller sample size from the HapMap project. They restricting the search to be for probes with known cis-acting eQTLs, and looking for interactions only between a cis-acting eQTL and all remaining trans-SNPs. Though it is unclear if any of these results surpassed a significance threshold that accounts for the experiment-wide testing burden, and no replication was attempted, it appears that there was enrichment for probes with nominally significant interactions. This is in stark contrast to the numerous additive effects that are typically observed in eQTL studies.103

Though we have only focused on a few examples here, the picture that is emerging is that compelling statistical support is absent for the vast majority of reported epistatic interactions. Potentially through some level of ascertainment bias, those epistatic effects that appear to be statistically robust have predominantly large marginal effects, and contribute relatively little in terms of phenotypic variance from interaction terms alone. Given the current empirical evidence, one can hypothesise that the number of instances of epistasis may indeed be large, but that the contribution of variance from gene interactions on complex traits is rather small.

## To which scientific question(s) is epistasis the answer?

### The missing heritability?

By definition, epistasis does not have a direct role to play in the problem of the missing heritability. The missing heritability pertains to the problem that there are insufficient additive effects discovered to account for the additive genetic variance estimated to exist for that trait. SNPs that interact can have marginal additive (and dominance) effects, but the nexus to declaring that they interact is that they have an epistatic effect that does not contribute to additive genetic variation. In principle, the total additive genetic variance accounted for by interacting SNPs can be captured by a standard, suitably powered GWA study searching for additive effects.

The metric of missing heritability emerges from two variables, the tally of genetic effects uncovered and the estimate of the trait's h2. In each case epistasis may have a subtle part to play. Firstly, some theoretical studies postulate that if, for some interacting loci, the epistatic variance is large compared to the additive variance, searching for epistatic effects might be one way to uncover loci with additive variance with more power than searching for the additive effects directly.115 Such instances might arise if the trait has a large **mutational target size** and is under direct selection.116 This scenario does not posit that epistatic variance contributes to additive variance, rather, searching for epistasis may lead to the identification of variants either rare or with small additive effects that would otherwise go undetected in a standard GWAS. Some empirical evidence exists to support this hypothesis,19,21 however it is unlikely to be a widespread phenomenon117 and theory shows that even in complex multi-locus epistatic patterns marginal additive effects are necessary to produce measurable additive variance for a trait.118

Secondly, because the direct estimation of non-additive genetic variation is almost always intractable, its contribution to the resemblance between relatives is unknown.1 Thus, it is possible that under certain experimental designs heritability estimates are inflated through contamination from non-additive variation.119 To what extent is this realistically a problem and how reliably can it be measured? A recent theoretical study gained much attention after demonstrating that redundancy amongst biological pathways could create an illusion of additive genetic variance in twin studies23 (provided that large common environmental effects were present120). Indeed it is a known issue in such study designs that, although it is not the most parsimonious model, a combination of non-additive variation and common environmental variation can lead to a significant additive parameter.121

To overcome this problem one can attempt to use family-based studies to estimate additive effects directly, and one can use contrasts between different types of relatives. For example, full siblings will share 0.5 additive variance and 0.25 dominance variance, while parent-offspring will share 0.5 additive and 0 dominance variance. If the correlation between degree of shared additive variation and phenotypic similarity is high across all types of relatives then this would be strong evidence for heritability estimates being uncontaminated by non-additive variance. Height shows consistent estimates of heritability between twin studies and family studies,122 but h2 estimates for BMI are much higher from twin studies than from family studies,123 suggesting that height is probably mostly influenced by additive effects but there is the potential for non-additive effects to play an important role in BMI.

### Elucidating putative biological mechanisms?

In principle, knowledge of genetically interacting regions could be very useful for two reasons. Firstly, like any associations, the actual locations of the variants can shed light on the mechanisms underlying the trait by virtue of being close to relevant genes or genomic features, and as discussed in the previous section theory suggests that in some situations, searching for epistasis directly may improve power to detect variants with small marginal effects.115,116,124

Secondly, the pattern by which they interact genetically can also be informative. For example, suppose an interaction is detected for a disease where risk is only conferred at the first polymorphism in the presence of the risk allele at the second polymorphism (for robust empirical examples of this pattern see 25,26,111). This may signify that there is pathway redundancy, and each variant affects independent pathways. An alternative pattern to the one described above, where the risk allele at one locus only has an effect in the absence of the risk allele at another locus, might suggest that both variants are involved in the same pathway because the loss of either variant is sufficient to confer the effect of the loss of the pathway. A potential example of this was shown in an interaction for systemic lupus erythratosus, where not only did the interaction replicate, but the genes involved (BLK and BANK1) were shown to co-localise *in vivo*.125

An extension of these ideas is to deconstruct complex traits into endophenotypes126 and analyse the endophenotypes themselves. It has been shown theoretically that if the endpoint of a metabolic pathway depends on the rate of expression of different enzymes within that pathway, then even if the rate of expression of each individual enzyme was controlled by purely additive genetic effects, then in many situations the genetic effects on the pathway outcome would appear non-additive.127 Further theoretical studies have shown that epistasis will form a large component of variation in pathway endpoints if there are negative feedback loops controlling the outcome.128

The limiting pathway model129 employs this idea to postulate that if a disease state depends on the states of multiple independent endophenotypes, where each effectively endophenotype120 has a polygenic additive genetic architecture, then there will be non-additive variance contributing to the disease state. Though such a model is biologically intuitive, it inherently specifies that power improvements can be made by modeling endophenotype networks and searching for additive effects within these lower level traits,130 rather than advocating the use of epistatic models to map genetic effects.

If epistasis were easily detectable in a hypothesis-free framework by an exhaustive genome-wide search, its potential to drive higher biological understanding would be very attractive. But because at this stage it appears that filtering or candidate gene approaches are often more likely to lead to the discovery of epistasis than hypothesis-free approaches, so far biological understanding has led to identifying epistasis (e.g. 112,131,132) more than epistasis has led to improving biological understanding.

### Evolution of complex traits?

The evolutionary mechanisms that lead to phenotypic variation in the population are wide ranging, and a purely additive framework can be used to parsimoniously explain the extant standing variation in human populations.133–137 Nonetheless, there is a large body of theory that discusses the role that functional epistasis plays in long term evolutionary models, with some compelling evidence of its existence in model organisms.138 But functional epistasis is of relatively low importance compared to **statistical epistasis** in dealing with phenotypic variation within populations. In this context, to what extent does evidence support the notion that epistasis is an important factor,138–140 and what is the consequence of epistasis influencing the evolution of human complex traits?

It has been argued that epistasis plays a role in different responses to genetic effects between species through the mechanism of Dobzhansky-Muller incompatibility,141,142 where non-synonymous mutations in humans exist with no adverse phenotype in other species, demonstrating a form of functional epistasis. For example, in humans a non-synonymous mutation causing the 53rd amino acid of alpha-synuclein from alanine to threonine predisposes to Parkinson’s disease. However, the homologous site in healthy mice and rats carries threonine, implying that there exist substitutions elsewhere that compensate for the effect. Such compensatory molecules can exist on the same molecule or on an entirely different molecule, and one study found 608 such examples of genetic suppression in a survey of 32 human proteins.143

Does this same mechanism arise within populations to influence complex traits through statistical epistasis? A compelling line of evidence has been shown by Lappalainen *et al*.144 in gene expression. Using sequence data, they showed that haplotypes carrying an allele that increased gene expression levels were significantly less likely to carry a putative functional coding variant in the gene due to allelic imbalance, suggesting that variants may be exposed to or masked from selection depending on their genetic backgrounds.116 In this case, it is clear that the evolution of complex traits are influenced by functional epistasis, but they will exhibit almost no statistical epistasis given the very low frequencies of the coding variants.

As shown in the example of the HLA-DR2 haplotype’s involvement in MS, another effect of selection acting on such local interactions is to prevent the decay of linkage disequilibrium across distant loci. It is difficult to ascertain the extent in which this occurs, because a) testing for LD being higher than expected by chance has very little power over short distances, b) it is very difficult to obtain orthogonal estimates of epistatic effects between SNPs in LD with each other,145 and c) the statistical effects across the population that would allow detection of such interactions are precisely the effects being minimized by selection in the first place. Nevertheless, it has been argued that such local interactions may indeed have an impact on the problem of the missing heritability. Haig (2011)33 theorised that in pedigree-based measures of heritability, haplotypes are essentially inherited as if they were multi-allelic blocks of DNA, such that the haplotype effect will contribute to heritability estimates, but if alternate haplotypes had opposing effects then on average each SNP will have no marginal additive effect.

### Genetic prediction?

A potential direct translation of large-scale genetic studies into medical practice is to use knowledge of an individual’s genetic profile to predict phenotypic outcomes.146 There are two main limiting factors in the accuracy of predicting phenotypes through genetic profiles. First, the maximum prediction accuracy is limited by the trait heritability.147 And second, the accuracy in which the genetic profile predicts the phenotype is dependent on the accurate estimation of the underlying genetic effects.148 In this context, is the inclusion of epistatic effects into genetic scores important for improving genetic prediction in complex traits?

With estimates of narrow-sense heritability obtainable for complex traits, one can estimate the maximum prediction accuracy under the assumption of perfect knowledge of all additive genetic effects. Conversely, our inability to estimate broad-sense heritability therefore makes it difficult to quantify the potential improvements that the inclusion of non-additive effects might incur. For highly heritable traits it is unlikely that non-additive genetic variance will form a large component of phenotypic variance, and therefore the inclusion of epistatic effects in genetic profile scores will not improve prediction accuracy. However one might speculate that non-additive variance could have a significant influence on the phenotypic outcome of more lowly heritable traits, and this may include endophenotypes such as methylation149,150 or gene expression levels.104

Another limiting factor in the inclusion of epistatic effects for genetic prediction is that, even assuming a significant non-additive genetic component, it is demonstrably hard to obtain reliable estimates of epistatic genetic effects. Nevertheless, it is not necessary to construct genetic predictors from significant effects as estimated through GWA studies alone. Using a relaxed threshold for the inclusion of additive effects into a genetic predictor has been shown to improve prediction accuracy for schizophrenia,151 demonstrating that an underlying polygenic architecture will comprise numerous small effects that fail reach a stringent significance threshold. Daetwyler *et al*.148 showed that in addition to heritability, the prediction accuracy is also a function of the ratio of the number of effects influencing the trait (often modeled as the number of independent markers in the genome) and the sample size.148,152 Therefore, for most complex traits that are polygenic in nature it is necessary to use extremely large sample sizes.153 In principle one could use this approach to also include epistatic effects, but because the effective number of independent pairwise genomic regions is dramatically higher154 than independent regions, a corresponding increase in sample size may be required to obtain gains in prediction accuracy equivalent to an additive model.

There has been use of epistatic effects for the prediction of complex traits in many model organism species. It was shown that including a network of epistatic effects in chicken lines155 for prediction in independent samples156 marginally improved prediction over the use of additive effects only. A number of reports have shown similar conclusions for several traits in plants also157–159, whereas others demonstrate that inclusion of epistatic effects yields no improvement in prediction accuracy,160 or that additive effects alone are sufficient to explain most genetic variation.161

## Conclusions

Though plenty remains to be done, a massive body of scientific discovery has been achieved through the additive genetic paradigm,162 in spite of its simplicity and seeming disconnection from realistic biological models. Although we can present a clear view on how to continue this progress (increase sample sizes, increase SNP density and rigorous standards of reporting (BOX 2)), it remains unclear to what extent epistasis will offer a solution to the major questions being posed about the genetics of human complex traits at this stage.

Nevertheless, the search for epistasis is fast becoming a relatively effortless one. Sophisticated computational techniques have made the analysis fast, interpretable, and potentially routine at the individual GWAS level. The next challenge is to greatly improve power of detection via meta analyses of multiple GWAS that are readily available. Therefore we believe that given this low cost to high potential benefit scenario the search for epistasis is indeed warranted.

## Box 1: Why is epistasis theoretically difficult to detect?

Supposing that non-additive variance is comprised of numerous small effects, as is the case for the additive genetic component, the statistical power to detect them is, in principle, much lower than that of detecting additive effects for a number of reasons. Note that these challenges to the detection of epistasis apply particularly to humans and other outbred species, it is possible that these challenges can be greatly reduced or even eliminated altogether in studies of model organisms163

##### Linkage disequilibrium

The variance explained by a SNP detected in a GWAS is unlikely to be equal to the variance explained by the true causal variant that is being tagged by the marker. The additive variance at the observed marker will decrease linearly with decreasing LD r2 between itself and the causal variant, thus if effect sizes are small then GWAS is dependent upon there being high LD between causal variants and observed SNPs. However, the dominance variance at the observed SNP reduces by a factor of r4, thus the dependence on high LD between observed SNPs and unobserved causal variants is much higher when detecting dominance effects. Extending this to two loci, the AxA variance on average reduces by r4 across both loci, the AxD variance by r6, and the AxD variance by r8. The consequence of these constraints is that any given SNP chip has substantially greater coverage of the genome when searching for additive effects than when searching for epistatic effects and indeed dominance. To overcome this problem one needs denser genotyping or high quality imputed genotypes or sequence data to identify non-additive effects at the same power as additive effects.

##### Curse of dimensionality

The search for additive effects is performed in a single dimensional search space, that is, our search is constrained to the number of markers in the experiment. In principle, searching for epistasis involves expanding from one dimension to two or more dimensions, thus the parameter space increases exponentially. This problem, where any signal becomes drowned out by the noise, is known as the "curse of dimensionality". There are several strategies that one can use to scan the genome for epistatic effects as discussed in the main text, but they typically involve expansion of the search space and a higher multiple testing penalty than is required for detecting non-epistatic effects. Therefore, in order to obtain the same power of detection as searching for additive effects, the sample size must increase and/or one depends on the epistatic variance being larger.

##### Model complexity

Modelling the additive effect of a SNP on a phenotype is achieved through a model that uses only one degree of freedom. However, for two loci there are four interaction terms (AxA, AxD, DxA, DxD), thus under the hypothesis that each of the four epistatic effects is equal in magnitude28,29,116 the model complexity for the statistical test increases. In hypothetically comparing the power to genetic loci of equal variance that comprise either additive effects or epistatic effects, the increased number of degrees of freedom used by the epistatic test will lead to a less extreme p-value despite capturing the same amount of variance as in the scenario for the additive test. The simple way to overcome this problem is to increase sample size.

##### Replication

Replication is key to confirming the statistical veracity of associations. The increased dependence of epistasis on high LD between observed SNPs and causal variants means that there is likely to be ascertainment in a discovery sample for markers in higher LD in the sample than they are in the population, simply due to sampling. Thus, taking an independent sample from the population it is one resamples the LD, and it is unlikely that the same ascertainment for high LD will occur. In this context we expect the replication rate of epistasis to be substantially lower than for additive effects. Performing detection and replication on very dense SNP chips, imputed genotypes, or sequence data will overcome this problem.

## Box 2: What constitutes a significant epistatic interaction?

Evidently, detection of epistasis is extremely challenging. But with sample sizes increasing rapidly, and the growing availability of high density SNP chips and sequencing, we are entering an era in which detection of epistasis may indeed be feasible. Here we suggest some considerations in how to report epistatic interactions with a view to the provision of robust conclusions that will be trusted by the scientific community.

##### Replication is necessary

As with additive genetic effects, following the discovery of epistatic genetic effects, the gold standard for reporting them is to show that the same SNPs replicate with the same direction effect on the phenotype in an independent sample.

##### Sufficiently stringent significance thresholds

If independent samples are not available for replication, stringent significance thresholds are of utmost importance. Based on there being approximately 1 million independent regions in the genome, GWAS studies have adopted a standard significance threshold of p = 5x10-8. With the emergence of rapid computational methods for performing exhaustive pairwise scans, we suggest a significance threshold of p = 0.05 / 0.5(1x106)2 = 1x10-13.

##### Discount the possibility of scale effects

Ideally quantitative analysis should be performed on phenotypes that are normalized through rank transformation, and it should be demonstrated that any detected non-additive effects persist following transformation of to a biologically relevant scale.164 For case-control traits the analysis of epistasis is most convincing when shown to be present on the liability scale of risk.

##### Discount the likelihood of haplotype effects

In a typical pairwise scan inflation of test statistics for interaction terms between neighbouring SNPs on the same chromosome can arise due to haplotype effects, and this is often inseparable from epistatic effects.21 Ideally the two tested SNPs should neither be in LD with each other nor should both be in LD with some other, potentially unobserved, variant. This latter caveat is impractical in the absence of sequence data and we suggest that interacting SNPs should be in LD r2<0.1, and D’<0.1 to reduce the possibility of haplotype effects underlying any signals.

## 

**Table 1**

Publicly available applications for fast genome-wide analysis of epistasis in GWAS\*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Type** | **Main feature** | **URL** | **Ref** |
| FastEpistasis | Regression | An efficient parallel extension to the PLINK epistasis module | <http://www.vital-it.ch/software/FastEpistasis/> | 10 |
| BOOST | Regression | Bitwise implementation, approximate tests for screening and logistic regression for testing | <http://bioinformatics.ust.hk/BOOST.html> | 19 |
| epiGPU | Regression | GPU-enhanced contingency table based proximate tests | <http://sourceforge.net/projects/epigpu/> | 8 |
| PIAM | Regression | Multithread parallel enabled, contingency table based LR tests?? | <http://www.ihs.ac.cn/xykong/PIAM.zip> | 9 |
| BiForce | Regression | Bitwise and multithread implementation, contingency table based proximate tests | <http://bioinfo.utu.fi/biforcetoolbox> | 16 |
| EPIBLASTER | LD | GPU-enhanced, LD-based screening and logistic regression for testing | <http://www.mpipsykl.mpg.de/en/epiblaster/index.html> | 15 |
| SIXPAC | LD | Bitwise implementation of Probably Approximately Complete search algorithm | <http://www.cs.columbia.edu/~snehitp/sixpac/> | 18 |
| GenomeMatrix | Haplotype | Haplotype-based odds ratio test for interactions | <https://sph.uth.edu/hgc/faculty/xiong/software-B.html> | 12, 33 |
| BEAM series | Bayesian | Bayesian partition model considering LD | <http://sites.stat.psu.edu/~yuzhang/> | 6 |
| BhGLM | Bayesian | Bayesian hierarchical Generalized Linear Model for haplotype interactions | <http://www.ssg.uab.edu/bhglm/> | 43 |
| SNPTEST | Bayesian | Bayesian model averaging approach to model interactions involving known risk loci | http://www.stats.ox.ac.uk/∼marchini/software/gwas/gwas.html | 46 |
| EDCF | Filtering | Clustering frequent genotype combinations for testing interactions | http://www.cs.ucr.edu/∼minzhux/EDCF.zip | 49 |
| RAPID | Data mining | Identify correlated SNP pairs after projecting their correlations to distance between two points in a Euclidean space | <http://bix.ucsd.edu/projects/rapid> | 51 |
| TEAM | Data mining | Using Minimum Spanning Tree incrementally updates the contingency tables for epistatic tests without scanning all individuals | <http://www.csbio.unc.edu/epistasis/> | 50 |

\*: Only list a small proportion of recent developed tools

## Figure 1

## Types of methods to detect epistasis in GWAS. Outline of different types of methods in two major groups based on SNPs and groups of SNPs respectively.

Regression

LD

Frequentist

Genome-wide

Haplotype

Partition

Hybrid

Bayesian

Knowledge

Filtering

Statistics

SNP-based

Algorithm

Machine learning

Artificial Intelligence

Gene

Data mining

Module

Group-based

## Glossary

**Complex trait**: A trait where variation between individuals is controlled by several or many genes and different environmental effects, potentially with interactions between these different effects.

**Genetic architecture**: The complete description of the genetic factors influencing trait variation, such as number of genetic loci, their effects, allele frequencies actions and interactions.

**Epistasis:** Statistical interactions between loci in their impact on a trait such that the impact of a particular single locus genotype depends on the genotype at other loci.

**Mutational target size:** Fraction of the genome in which new mutations potentially cause variation for a trait. For most complex traits this is large suggesting many loci can potentially influence trait variation.

**Mendelian disease**: Where the disease state is completely or largely determined by variation at a single gen locus inherited in a Mendelian fashion.

**Heritability**: The proportion of the trait variation for a particular trait in a particular population and environment that is under genetic control. The **narrow-sense heritability (h2)** refers to the proportion of variation due to the additive effects of genes whereas the **broad-sense heritability (H2)** is the proportion due to all genetic effects.

**Marginal effect:** the average effect of a locus across all other loci and environmental effects

**Hypothesis free analysis:** No assumption is made about the loci involved in epistasis or their effects and so all possible pairs of SNPs are tested (an **exhaustive search**). **Hypothesis driven analysis** limits the combinations of loci tested according to some prior hypothesis (for example that only loci with a marginal effect should be tested or only those loci in a particular pathway).

**Endophenotype:** A heritable trait genetically correlated with a disease trait. Often a trait that be measured in all individuals whether diseased or not and which potentially provides a predictor of disease status.

**Polygenic architecture:** A trait genetic architecture under which many genes of small effect contribute to trait variation.

**Binary phenotype**: Disease traits often have two major states, diseased or healthy. They may nonetheless be complex traits where transition to the disease state is influenced by an underlying **liability** to disease that is controlled by many genetic loci and environmental effects.

**eQTL:** An expression quantitative trait locus controls variation in expression of a particular gene. An eQTL may lie adjacent to the gene being controlled (*cis* acting control) or some distance away (*trans* acting).

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