Genome analysis

Using tree-based methods for detection of gene–gene interactions in the presence of a polygenic signal: simulation study with application to educational attainment in the Generation Scotland Cohort Study

Joeri J. Meijsen1,2, Alexandros Rammos3, Archie Campbell1, Caroline Hayward4, David J. Porteous1,2, Ian J. Deary2,5, Riccardo E. Marioni1,2 and Kristin K. Nicodemus1,2,*

1Centre for Genomic and Experimental Medicine, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XY, UK, 2Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh EH8 9JZ, UK, 3Department of Genetics, Smurfit Institute of Genetics and Institute of Neuroscience, Trinity College Dublin, Dublin D02 VH29, Ireland, 4MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XY, UK and 5Department of Psychology, University of Edinburgh, Edinburgh EH8 9JZ, UK

*To whom correspondence should be addressed.

Abstract

Motivation: The genomic architecture of human complex diseases is thought to be attributable to single markers, polygenic components and epistatic components. No study has examined the ability of tree-based methods to detect epistasis in the presence of a polygenic signal. We sought to apply decision tree-based methods, C5.0 and logic regression, to detect epistasis under several simulated conditions, varying strength of interaction and linkage disequilibrium (LD) structure. We then applied the same methods to the phenotype of educational attainment in a large population cohort.

Results: LD pruning improved the power and reduced the type I error. C5.0 had a conservative type I error rate whereas logic regression had a type I error rate that exceeded 5%. Despite the more conservative type I error, C5.0 was observed to have higher power than logic regression across several conditions. In the presence of a polygenic signal, power was generally reduced. Applying both methods on educational attainment in a large population cohort yielded numerous interacting SNPs; notably a SNP in RCAN3 which is associated with reading and spelling and a SNP in NPAS3, a neurodevelopmental gene.

Availability and implementation: All methods used are implemented and freely available in R.

Contact: kristin.nicodemus@igmm.ed.ac.uk

Supplementary information: Supplementary data are available at Bioinformatics online.
1 Introduction

Historically, genomic association studies have focused almost exclusively on single-loci and/or polygenic risk score (PGRS) associations. These methods have been very successful; however, frequently they do not explain the total genetic variance of a trait estimated by twin studies. Therefore, it is also important to consider non-additive genetic effects such as epistasis in the complex genetic architecture of human traits. Epistasis has been described as one genetic locus masking or modifying alleles of other loci (Bateson and Mendel, 1909) or a deviation from additivity of two genetic variants on a phenotypic trait (Fisher, 1919). Epistasis, in the sense of ‘deviation from additivity’ can be defined as either antagonistic (a model where the interaction decreases or blocks the effect of each individual allele) or synergistic (where a combination of alleles exacerbates the effect of each allele individually). Many—if not most—complex traits might have different components of genomic architecture of varying importance—e.g. strongly associated single SNPs, a polygenic component and an epistatic component. The evaluation of statistical learning methodologies for the detection of these different components, to our knowledge, has not been performed.

Even though epistasis has been observed and is well documented in multiple non-human organisms (Brockmann et al., 2000; Cheng et al., 2011; Grice et al., 2015; He et al., 2016; Huang et al., 2012; Mackay, 2014), whether or not epistasis exists and plays a vital role in human traits remains an open debate (Hill et al., 2008; Huang and Mackay, 2016; Mackay and Moore, 2014; Sackton and Hartl, 2016; Webber, 2017). According to the ‘omnigenic’ model, in complex traits the disease-related genetic signal tends to be spread across the genome, resulting in genes without direct statistical association to the trait. Therefore, the ‘omnigenic’ model states that, due to a large interconnection between gene regulatory networks, most heritability can be explained by the surrounding genes outside the core disease-related genes, which likely includes epistasis (Boyle et al., 2017). In general, human epistatic studies have shown limited success, partially due to the use of restrictive methods such as searching within subsets of loci or for specific SNP interaction sizes (e.g. hypothesis-driven analysis) in order to lower the number of tests that need to be performed and thus the resulting statistical correction that has to be applied.

Recently, increasing efforts have been placed on addressing the statistical and computational problems related to the detection of epistasis in large datasets. Machine learning (ML) algorithms are increasingly used to ascertain classifiers for either data reduction or feature selection. These include tree-based methods like random forest (RF), classification and regression trees (CART) (Chen et al., 2011; García-Magariños et al., 2009; Stephan et al., 2015) and likelihood ratio Mann-Whitney tests (Lu et al., 2012). García-Magariños et al. (2009) simulated genotype data containing interacting SNPs under multiple scenarios (sample size, missing data, minor allele frequencies and several penetrance models). This study found that CART and RF were equally good in detecting interacting SNPs. Even though the study simulated 99 different scenarios with 100 replicates each, the simulated datasets are very small (two ‘causal’ SNPs plus 98 null SNPs) and do not reflect the scale or complexity of modern genomic studies.

We sought to apply greedy non-parametric decision tree-based methods—C5.0 and logic regression—for the detection of epistasis in large-scale studies, as these methods explicitly model interactions. C5.0 constructs rule-based decision trees using solely the Boolean operator OR (Fig. 1A) whereas logic regression allows for Boolean operators AND, OR and NOT (Fig. 1B). Note that logic regression is a regression framework therefore allowing for the construction of multiple trees (e.g. multiple trees acting as predictors in a regression model), whereas C5.0 constructs multiple rulesets and is not embedded in a regression framework. To date, C5.0 has not been applied to genetic data in the search for interactions, whereas logic regression has been shown to be effective in detecting main effects and interactions in genetic data and could be used as a comparison method (Chen et al., 2011; Kooperberg et al., 2001; Ruczinski et al., 2003, 2004; Schwender and Ickstadt, 2008).

We sought a complex, but well studied trait to test these approaches. Educational attainment (EA) is a highly heritable complex trait (Calvin et al., 2012; Krapohl et al., 2014) and is highly influenced by social and other environmental factors; however, SNP-based heritability estimates that genetic factors contribute to around 20% of variation across individuals, while average twin-based heritability is around 40% (Rietveld et al., 2013). The largest GWAS to date investigating years of education as a proxy of EA observed 74 statistically significant SNPs (Okbay et al., 2016) of which 72 were replicated in the same study using the large UK Biobank cohort. PGRS derived from the same GWAS explained 3.9% of the variance in years of education in an independent sample. This large gap of missing heritability (Δh²=μ−h²SNP) is in similar to that found in other complex traits, however the moderate correlation with traits showing evidence of epistatic contribution e.g. personality traits (Jang et al., 1996; Loehlin et al., 2003; de Moor et al., 2012; Power and Pluess, 2015; Vukasović and Bratko, 2015) hints towards an epistatic contribution.

In this study, we applied C5.0 and logic regression on simulated epistatic data under multiple scenarios to show their capability of detecting interacting loci in a large genetic study. We sought to assess the performance of C5.0 and logic regression to detect epistatic components alone, plus in the presence of a polygenic signal in order to inform about the methodological development of models that include effects of single SNPs, additive or polygenic components as well as epistasis. To our knowledge, this will be the first simulation study to date to examine the detection of epistasis in the presence of a strong polygenic signal. We applied both methods on the genome-wide SNP data from the Generation Scotland: the Scottish Family Health Study (GS: SFHS) cohort to investigate whether there is evidence for an epistatic contribution to years of education as a measurement of educational attainment.

2 Materials and methods

2.1 Statistical methodology

2.1.1 Classification and regression trees (CART)

CARTs are decision tree-based methods that can be interpreted as a set of decisions leading along a path to a final prediction. CART

![Fig. 1. Visual representation of a C5.0 and logic tree. (A) C5.0 decision tree; (B) logic tree](image-url)
methods utilize classifiers (measurements) to ‘split’ the data into partitions. CART methods solely use the Boolean operator OR to split a classifier (e.g. male OR female). CART methods grow a tree by including classifiers (recursive partitioning), calculating for every split the ‘impurity’ or misclassification rate, and define a split with the lowest impurity. Commonly-used impurity measurements are the Gini index for classification-based methods and sum of squared residuals for regression-based methods. CART methods keep recursively partitioning the dataset until no split that decreases impurity can be made or when the size of the terminal nodes (e.g. subjects in node) is less than some user-defined value or is 1. This most often leads to a large tree where some terminal nodes only contain a small number of individuals. The complexity of a tree can be decreased by pruning sections of the tree that provide little power to classify observations.

2.1.2 C5.0 and logic regression

C5.0 is a modified version of Quinlan’s non-parametric C4.5 classification algorithm (Quinlan, 1992). C5.0 builds decision trees, performs rule-based models and evaluation of variable importance (Kuhn and Johnson, 2013; Wu et al., 2008). C5.0 decision trees are built by using information entropy (1).

\[ \text{info}_i^{\text{before}} = - \sum_{i=1}^{m} p_i \log p_i \]  

where \( p_i \) is the probability of a given class \( i \) as the outcome for each of \( m \) possible classes and \( S \) is the split.

To build a tree containing optimal splits, C5.0 assesses, for each node, the normalized information gain which acts as the purity criterion. For each node C5.0 calculates the information entropy before (1) and after (2) a split.

\[ \text{info}_i^{\text{after}} = - \sum_{i=1}^{K} \frac{n_i}{n} \log \frac{n_i}{n} \]  

where \( S \) is the split; \( K \) is the number of partitions; \( n_i \) is the number of samples \( i \) assigned to partition \( K \); \( n \) is the total number of samples and \( \text{info}_i \) is the sum of the information entropy in the \( r \)th resulting partition.

For a given node with split \( S \) and \( K \) partitions, C5.0 calculates the information entropy for each resulting partition. This is subsequently multiplied by the proportion of samples assigned to that partition \( \frac{n_0}{n} \). This adds a weight to each partition, which is summed over all partitions resulting in the information entropy after split \( S \). A lower information entropy after the split implies an information gain (positive difference) and therefore a decrease in uncertainty. If entropy increases (negative difference) C5.0 stops adding splits. The information gain is normalized to allow for the consideration of each class. C5.0 then selects the class with the highest normalized information gain. This process is repeated recursively for smaller subsets.

Each top-to-bottom path in the final tree is collapsed into a so-called rule. C5.0 evaluates each rule on independent conditional statements, thereby assessing whether or not they can be generalized by removing terms in the conditional statement. This process is called rule-based pessimistic pruning and in short removes branches that are not contributing to the improvement of the trees classification. As a final step, C5.0 assigns each rule to a class by calling a vote. The class with the highest vote is used. Results in a single pruned tree where each possible combination from the top node to bottom node in the tree is a so-called ruleset.

Logic regression is a non-parametric adaptive regression method (Ruczinski et al., 2003). Logic regression is largely based on the same principles as a CART, but in contrast to CART, logic regression constructs logic trees \((L)\). Logic trees are Boolean combinations (AND, OR and NOT) of binary predictors e.g. \( L_1 = \text{SNP}_1 \) or \( [\text{SNP}_1 \text{ and NOT SNP}_2] \). This increases the complexity compared to CART which solely applies the Boolean operator OR. A logic tree can be used as a predictor in a regression model (3). Due to its adaptive nature, logic regression estimates the coefficients \( (\beta) \) and Boolean expressions \( (L) \) at the same time.

\[ y = \beta_0 + \beta_1 L_1 + \beta_2 L_2 + \cdots + \beta_p L_p \]  

By doing so, logic regression tries to minimize the scoring function associated with a model type (e.g. residual sum of squares for quantitative outcomes). For the construction of logic trees, logic regression starts at a random starting point and applies a greedy hill climbing algorithm, which keeps adding predictors to the model as long as the misclassification rate goes down and only stops when the misclassification rate goes up.

2.2 Simulation and genetic methodology

2.2.1 Generation Scotland

Generation Scotland: the Scottish Family Health Study (GS: SFHS) is a large, family-based cohort study sampled from the general population in Scotland (www.generationscotland.org). The study design has been widely documented (Smith et al., 2006, 2013). In short, 24,000 individuals were recruited in the study during a five-year period (2006–2011). The individuals were deeply phenotyped for a wide variety of traits such as lifestyle factors, family history and health outcomes. DNA of 20,128 GS: SFHS individuals were analyzed by means of high density genome wide bead array genotyping (Illumina OmniExpress 700K SNP GWAS and 250K exome chip). DNA results of 134 individuals were excluded during quality control leaving 19,994 genotyped individuals.

We removed single nucleotide polymorphisms (SNPs) and individuals >5% missing data and removed SNPs with a minor allele frequency <1%. We used Genome-wide Complex Trait Analysis (GCTA) (Yang et al., 2011) to extract a list of genetically-unrelated individuals, giving a total of 7372 individuals (relatedness < 0.025, corresponding to second degree cousins). For the simulation study, we selected 5000 individuals at random from the unrelated set.

We selected the gene-rich chromosome 19 (10,756 SNPs) for analysis. Using PLINK (Purcell et al., 2007) we performed linkage disequilibrium (LD) pruning on chromosome 19 (window size = 50 kb, step size = 5 kb and \( r^2 \) threshold = 0.1), leaving 1705 SNPs in linkage equilibrium (LE). From the LD pruned dataset we designated the potential set of ‘causal’ SNPs in a minor allele frequency range of 0.4–0.5 (584 SNPs). From this pool, high minor allele frequency SNPs were selected to ensure equally high levels of statistical power across all simulations. All analyses were performed twice: once on the linkage disequilibrium pruned (1705 SNPs) and again on the unpruned (10,756 SNPs) chromosome 19 datasets.

2.2.2 Simulation of phenotypes

We simulated phenotypes under the alternative hypothesis \((H_1)\) and null hypothesis \((H_0)\) with 500 replicates per condition. All added errors \((e)\) were drawn from a standard normal distribution \( N (\mu = 0, \sigma^2 = 1) \). To ensure unbiased simulation, bias calculations were performed to assess possible over/under estimations of coefficients. Coverage was calculated to assess the probability that the sum of the
estimated coefficients fell in the 95% confidence interval using a regression model.

**Polygenic phenotype**

We selected 200 SNPs from the potentially causal SNP pool to form a polygenic phenotype. In this model each SNP explains the same amount of variation ($R^2 = 1.5 \times 10^{-3}$) with a total $R^2$ of 0.3 (30%) (4). Simulations were performed using the Linkage-Disequilibrium Adjusted Kinships (LDAK) software (Speed et al., 2012). Bias was observed at 0.18 and coverage was 96%. The bias calculation in the polygenic phenotype is larger compared to other phenotypes.

$$y = \beta_1 SNP_1 + \beta_2 SNP_2 + \beta_3 SNP_3 \ldots + \beta_2SNP200 + \epsilon \quad (4)$$

**2-SNP interacting phenotypes**

Two SNPs not used for simulating the polygenic phenotype were selected at random from the potentially causal SNP pool. We simulated 2-SNP interacting phenotypes assuming each individual SNP has a small but present main effect ($\beta_1 \neq 0$ and $\beta_2 \neq 0$) (5).

$$y = \beta_1 SNP_1 + \beta_2 SNP_2 + \beta_3 SNP_1 \cdot SNP_2 + \epsilon \quad (5)$$

We simulated three levels of 2-SNP interactions (weak, intermediate and strong) each explaining a different amount of variation (Table 1). The weak interaction phenotype strength was simulated to represent an interaction that would not be detected by a regression model after adjusting for multiple testing by means of a Bonferroni correction (mean $P$-value = $3.1 \times 10^{-2}$; median $P$-value = $1.8 \times 10^{-3}$). The strong interaction phenotypes had a mean $P$-value = $1.3 \times 10^{-10}$ and median $P$-value = $3.5 \times 10^{-17}$ to assess whether C5.0 and logic regression were capable of detecting a strong signal; this phenotype was used as a proof of principle. Intermediate phenotypes (mean $P$-value = $3.6 \times 10^{-4}$; median $P$-value = $2.0 \times 10^{-7}$) were simulated to fall between the two extremes (Table 1). Bias calculations were all close to 0 (strong = $4.0 \times 10^{-3}$, intermediate = $6.7 \times 10^{-4}$ and weak = $-5.0 \times 10^{-7}$) and coverage was 96% for the strong phenotype and 94% for both the intermediate and weak phenotypes.

**3-SNP interacting phenotypes**

Three SNPs not previously used for simulating the polygenic phenotype were selected at random from the potentially causal SNP pool. We analyzed these phenotypes independently. Three levels (weak, strong and pure) of 3-SNP interactions were simulated including all possible 2-SNP interactions (6).

$$y = \beta_1 SNP_1 + \beta_2 SNP_2 + \beta_3 SNP_3 + \beta_4 SNP_2 \cdot SNP_3 + \beta_5 SNP_1 \cdot SNP_3 + \beta_6 SNP_1 \cdot SNP_2 + \beta_7 SNP_1 \cdot SNP_2 \cdot SNP_3 + \epsilon \quad (6)$$

We simulated a weak and strong 3-SNP interacting phenotype explaining a different amount of variation; we set the $\beta$s of the strong interaction to be twice as large as the weak ones. (Table 2). Also, we simulated a pure 3-SNP interaction where in Equation 6 $\beta_1$ to $\beta_6$ are all set to 0. Bias calculations were again all close to 0 (pure = $-7.41 \times 10^{-4}$, strong = $2.26 \times 10^{-4}$ and weak = $-5.87 \times 10^{-4}$) and coverage was 97% for the pure phenotype and 93% for both the intermediate and weak phenotypes.

**Combined polygenic and interacting phenotypes**

To assess the capability of C5.0 and logic regression to detect gene-gene interactions even in the presence of an additive or polygenic component we simulated an interaction in the data used for the polygenic simulations, using SNPs not included in the polygenic component.

**Null phenotype**

To assess the type I error, we modelled a phenotype under $H_0$ where all $\beta$s are set to 0; therefore, $y = \epsilon$. Bias was observed as $1.2 \times 10^{-3}$ with a coverage of 94%.

**Main effect**

To rule out the possibility that the power was driven by the larger $\beta$s of the main effects within the interaction phenotypes, we simulated 500 replicates that only included the main effect of the strong phenotype; $y = \beta_1 SNP_1 + \beta_2 SNP_2 + \epsilon$ (where $\beta_1$ and $\beta_2 = 0.2$). As this interaction has the largest coefficients we chose this setting as a proof of principle for all other phenotypes with smaller coefficients. Bias was observed as $9.0 \times 10^{-3}$ and coverage of 96.4%. The strong two-SNP main effect signal was then combined with the 200-SNP polygenic signal.

**2.2.3 Data pre-processing and parameter settings**

Genotype data were converted to ordered vectors. Logic regression only allows for binary predictors; therefore, we dichotomised the genotype data into dominant and recessive predictors, i.e. genotype [0, 1, 2] becomes dominant [0, 1, 1] and recessive [0, 0, 1]. Missing genotypes were imputed by means of median imputation before analysis.

**2.2.4 Calculating type I error and power**

We defined the type I error for C5.0 as the percentage of trees constructed under $H_0$. Power was defined as the percentage of constructed sets of rulesets under $H_1$ containing all of the simulated interacting SNPs.

For logic regression, we assessed the presence of a signal under $H_0$ and $H_1$ by performing a randomisation test. Each replicate was permuted 100 times; the number of instances the original model had a lower score (residual sum of squares) than 95% of permuted models ($x = 0.05$) was derived. For type I error, we counted the number of times that the replicate passed the randomisation test when no signal was present and divided by 500. For power, we considered only those replicates that passed the randomisation test, and similarly for the calculation for type I error (Fig. 2A). Then we assessed if the logic trees contained all the simulated interacting SNPs. If the replicate (a) passed the randomisation test and (b) the simulated interacting SNPs were present, this was considered as a ‘true positive’ and we summed the number of these replicates and divided by 500 to obtain power (Fig. 2B).
weak interaction analyses C5.0 did not detect a single ruleset in 98.8% of the replicates. In the remaining 1.2%, C5.0 was not able to detect both interacting SNPs (Supplementary Table S1). Higher order interactions, i.e. 3-SNP interaction, were also detected using C5.0. We observed a power of 100, 100 and 90.4% of all three interacting SNPs in the pure, strong and weak 3-SNP interaction phenotypes (Supplementary Table S2). When combining these phenotypes with a polygenic signal, the interaction power remained 100% for the pure and strong phenotypes and dropped to 11.2% for the weak phenotype (Supplementary Table S2). For replicates that only contained two SNPs with main effects and no interaction, we observed that C5.0 detected 62.6% rulesets containing solely one of the two main effect SNPs, in 32.2% both SNPs and in 2.2% no rulesets. When combined with a polygenic signal this dropped to 0.4% for both SNPs and one of the main effect SNPs in 16% while it did not detect any ruleset in the remaining 84%.

Randomisation test-based analyses showed the power of logic regression using the LD pruned data ranged between 89.6% (combined polygenic and weak 3-SNP interaction) and 100% (Table 4) for phenotypes containing interactions and 99.6% for the polygenic-only phenotype. For each of the 500 polygenic replicates logic regression created a model containing eight SNPs. These models contained either 2 (1%), 3 (2.6%), 4 (11%), 5 (24.7%), 6 (31.1%), 7 (22.9%) or 8 (6.6%) polygenic SNPs. This means that, in all replicates minus the 6.6% containing 8 polygenic SNPs, logic regression includes several SNPs that can be defined as false-positives when a polygenic signal is present because these SNPs have been LD-pruned; thus, their presence is not due to correlation with a polygenic SNP.

For all 2-SNP interaction analyses logic regression created trees containing eight SNPs with the exception of ten trees (0.15%) containing 1 (1 tree), 3 (1 tree), 4 (2 trees), 5 (2 trees), 6 (1 tree) or 7 (3 trees) SNPs. For the strong and intermediate 2-SNP phenotypes, logic regression created in 99.8 and 98.8% replicates logic trees containing both interacting SNPs (Supplementary Table S3). Several of the remaining SNPs in these trees were false positives, not due to LD. This dropped to 77% in the weak 2-SNP phenotype, with 1% containing no interacting SNPs. When combining the polygenic and epistatic phenotypes the trees contained the interacting SNPs in the strong (98.8%), intermediate (82.8%) and weak (9.6%) phenotypes (Supplementary Table S3). Furthermore, 45.3% of the created combined polygenic-weak trees contained no interacting SNPs. The majority of trees in the higher order 3-SNP interaction analyses contained the interacting SNPs (pure 99.6%, strong 99.6% and weak 89%; Supplementary Table S4a). This number decreased when adding the polygenic component (pure 98.4%, strong 95.2% and weak 53.6%; Supplementary Table S4b). No trees were observed containing solely non-interacting SNPs.

### Table 2. Three-SNP interaction models, $R^2$ and $p$-values

| Model | $\beta_1, \beta_2, \beta_3$ | $\beta_4, \beta_5, \beta_6$ | $\beta_7$ | $R^2$ (interaction) (%) | $R^2$ (full model) (%) | Mean $p_{\text{interaction}}$ | Median $p_{\text{interaction}}$ |
|-------|----------------|-----------------|-------|----------------|--------------------|------------------|------------------|
| Pure  | 0   | 0               | 0.4   | 0.04           | 1.86               | 30.1             | $1.0 \times 10^{-14}$ | $1.1 \times 10^{-12}$ |
| Strong| 0.05| 0.1             | 0.2   | 0.19           | 0.41               | 39.9             | $4.5 \times 10^{-4}$   | $6.6 \times 10^{-7}$   |
| Weak  | 0.025| 0.05           | 0.1   | 0.15           | 0.1                | 14.3             | $7.7 \times 10^{-2}$   | $1.3 \times 10^{-2}$   |

### Fig. 2. Flow chart for logic regression analyses

### 3 Results

#### 3.1 Type I error

We observed that C5.0 has a type I error of 0% when using the LD pruned data and 0.6% when using the LD unpruned data. Using the randomisation test we observed that logic regression has a 5.8% type I error using LD pruned data, rising to 6.4% when using LD unpruned data.

#### 3.2 Power

##### 3.2.1 LD-pruned data

Power results for pruned and unpruned data for both methods can be found in Table 3. C5.0 detected rulesets in 11.4% polygenic replicates with the LD pruned dataset. Of these, 79% were based on a single SNP, 19% on two SNPs and 2% on four SNPs. All observed SNPs in the rulesets were from the 200 SNPs used to create the polygenic phenotypes, with no un-associated SNPs in any rulesets. C5.0 detected the two interacting SNPs in 100% of the strong replicates. This number decreased to 99.2% in the intermediate and 8.6% in the weak replicates (Supplementary Table S1). No rulesets were created that included other non-interacting SNPs; in other words, no false-positive SNPs were included in any of the rulesets generated for the interaction simulation replicates. Furthermore, in 35% of the weak replicates no ruleset was created; the remainder contained just one of the two interacting SNPs. In the combined polygenic and 2-SNP interaction phenotype analysis, C5.0 shows that it is capable of distinguishing additivity from interactions by detecting the two interacting SNPs in 100% of strong and 23% of intermediate replicates (Supplementary Table S1). In the combined polygenic and
polygenic SNP ($r^2 > 0.25$). Compared to the pruned set analyses, the percentage accurately detecting 2-SNP interactions by C5.0 remained 100% for the strong phenotype but decreased to 98.2% in the intermediate phenotype. The percentage accurately detected 2-SNP interactions increased to 19.8% in the weak phenotype (Supplementary Table S6). However, it has to be noted that C5.0 detected 3.2% (16 rulesets) non-interacting random SNPs of which 12 contained SNPs in LD with the true signal ($r^2 > 0.25$; this threshold was set to be consistent with the value for LD pruning). In the combined polygenic and interaction phenotype analysis, the power remained unchanged for the strong phenotype. The power was again higher in the intermediate (41.6%) and weak (0.6%) phenotype, but 10.6 and 6.6%, of replicates respectively, contained at least one false positive SNP (Supplementary Table S6). This dropped to 1.4% when taking LD structure into account ($r^2 > 0.25$). The rest of the trees contained either 1 (33.4%), 2 (29.6%), 3 (14.6%), 4 (5.6%), 5 (1%) or 6 (0.4%) polygenic SNPs and in 89.3% in combination with numerous SNPs in LD with the polygenic SNPs. The power of logic regression for the two interacting SNP phenotypes was 52.8% in the strong, 17.6% in the intermediate and 0.3% in the weak phenotype (Supplementary Table S8). This dropped further in the combined analysis to 23.4% in the strong, 2.2% in the intermediate and 0% in the weak phenotype (Supplementary Table S8).

We observed that in the higher order phenotypes, the trees contain three forms of the interacting SNPs is 50.2% (pure), 35.4% (strong) and 14.7% (weak) (Supplementary Table S9a). In line with previous observed results when adding the polygenic signal, the numbers again lowered to 35.2% (pure), 21% (strong) and 2.2% (weak) (Supplementary Table S9b). For all phenotypes a percentage of trees were created containing non interacting SNPs; however, the majority of these trees contained SNPs in LD ($r^2 > 0.25$) with the interacting SNPs (for a detailed outline see Supplementary Table S10).

### 3.3 Application to educational attainment in GS: SFHS

Having assessed our methods by simulation, we wished to test the approach on a large set of complex trait data. We extracted 7012 unrelated GS: SFHS individuals of which 6765 individuals had a measure of years of education, measured by ordered categories (e.g. 0: 0 years, 1: 1–4 years, 2: 5–9 years). We performed a linear regression analysis between years of education controlling for sex and age, and extracted the residuals to act as an adjusted years of education measurement (Zhao et al., 2012). Finally, we applied C5.0 and logic regression on the residual years of education outcome using 131821 whole genome SNPs in LE (LD pruning settings; window size = 50kb, step size = 3kb and $r^2$ threshold = 0.1). C5.0 detected 32 rulesets associated with educational attainment containing in total 30 SNPs (Supplementary Table S11). The logic regression model did not pass the randomisation test ($z = 0.05$) so will not be discussed further.

### 4 Conclusions and discussion

When using LD-pruned genetic data we observed that C5.0 is capable of distinguishing additivity from interactions. C5.0 created

---

Table 3. Power of C5.0 and logic regression in pruned and unpruned data, with and without polygenic component

| Condition                  | C5.0: – polygenic | LR: – polygenic | C5.0 + polygenic | LR + polygenic |
|----------------------------|-------------------|-----------------|------------------|----------------|
| 2-SNP, Pruned, Weak        | 8.6%              | 77.0%           | 0%               | 9.6%           |
| 2-SNP, Pruned, Intermediate| 99.2%             | 96.8%           | 23%              | 82.3%          |
| 2-SNP, Pruned, Strong      | 100%              | 99.8%           | 100%             | 98.8%          |
| 2-SNP, Unpruned, Weak      | 19.8%             | 0.3%            | 0.6%             | 0%             |
| 2-SNP, Unpruned, Intermediate| 98.2%            | 17.6%           | 41.6%            | 2.2%           |
| 2-SNP, Unpruned, Strong    | 100%              | 52.8%           | 100%             | 23.4%          |
| 3-SNP, Pruned, Weak        | 90.4%             | 89.0%           | 3.6%             | 53.6%          |
| 3-SNP, Pruned, Strong      | 100%              | 99.6%           | 100%             | 95.2%          |
| 3-SNP, Pruned, Pure        | 100%              | 99.6%           | 100%             | 98.4%          |
| 3-SNP, Unpruned, Weak      | 91.0%             | 14.7%           | 24.0%            | 2.2%           |
| 3-SNP, Unpruned, Strong    | 100%              | 35.4%           | 100%             | 21.0%          |
| 3-SNP, Unpruned, Pure      | 100%              | 50.2%           | 100%             | 35.2%          |

LR, Logic Regression.

Table 4. Power of logic regression based on randomization tests

| Model                          | Power: Pruned | Power: Unpruned |
|-------------------------------|--------------|-----------------|
| Weak 2-SNP interaction        | 97.4         | 64.6            |
| Intermediate 2-SNP interaction| 100          | 100             |
| Strong 2-SNP interaction      | 100          | 100             |
| 30% Polygenic + Weak 2-SNP interaction | 89.6         | 54              |
| 30% Polygenic + Inter. 2-SNP interaction | 100         | 89.6            |
| 30% Polygenic + Strong 2-SNP interaction | 100         | 100             |
| Weak 3-SNP interaction        | 100          | 99.2            |
| Strong 3-SNP interaction      | 100          | 100             |
| Pure 3-SNP interaction        | 100          | 100             |
| 30% Polygenic + Weak 3-SNP interaction | 100         | 92.6            |
| Polygenic + Strong 3-SNP interaction | 100         | 100             |
| Polygenic + Pure 3-SNP interaction | 100         | 100             |
| Polygenic model               | 99.6         | 94.4            |
rule sets observed under $H_0$; we conclude that C5.0 had a Similar results were observed in the 3-SNP combined analyses. As spurious results when the interaction term is of low magnitude. ruleset in 98.8% of the replicates showing it to be protective against nal in 100 and 23% of the strong and intermediate 2-SNP phenome was able to distinguish the interaction signal from the polygenic sig- Table S12). The main strength of this study is that we assessed the capability of both C5.0 and logic regression in detecting simulated genetic interactions under a wide range of settings including a strong polygenic signal. We suggest that C5.0 rulesets might be used as predictors within a regression model alongside single SNPs and additive or polygenic components (Nicodemus et al., 2014). The same can be done with logic trees. Limitations lie in the modest sample size ($N=5000$) and the use of only causal SNPs with a large MAF (0.4–0.5). We did not simulate phenotypes containing multiple SNP inter- actions (polygenic-epistatic phenotype) which is biologically plausible. In conclusion, we have shown that C5.0 and logic regression are capable of detecting simulated genetic interactions in a wide range of association levels and even in the presence of a strong polygenic component. We showed that when applying both methods LD pruning helps by improving the power and reducing the type I error. Finally, using C5.0 we were able to detect 32 rulesets containing 30 SNPs not previously reported with EA in Generation Scotland; RCAN3 has been previously observed in association with learning and reading while NPAS3 is involved in neurodevelopment. These methods are capable of detecting SNPs not directly associated to the trait but rather in sets of SNPs that together affect the trait. These methods are well-adapted to testing hypotheses regarding the ‘omnigenic’ model.

Acknowledgements
We wish to thank the participants of Generation Scotland.

Funding
JJM was partially supported by Rosetrees Trust Biomedical Research Grant M405 to KKN. AR and KKN were supported by the Irish Research Council GOPiG2013/763. SER, JM and KKN were supported by a University of Edinburgh Chancellor’s Fellowship. Generation Scotland received core sup- port from the Chief Scientist Office of the Scottish Government Health Directorates [CZD/16/6] and the Scottish Funding Council [HR03006]. Genotyping of the GS: SFHS samples was carried out by the Genetics Core Laboratory at the Edinburgh Clinical Research Facility, University of Edinburgh, Scotland and was funded by the Medical Research Council UK and the Wellcome Trust (Wellcome Trust Strategic Award ‘STratifying Resilience and Depression Longitudinally’ (STRADL) Reference 104036/Z/ 14/Z). IJD is supported by the Centre for Cognitive Ageing and Cognitive Epidemiology, which is supported by the Medical Research Council and the Biotechnology and Biological Sciences Research Council (MR/K026992/1). GS: SFHS genotyping was funded by the Medical Research Council UK through the MRC Human Genetics Unit QTL in Health and Disease core programme.

Conflict of Interest: none declared.

References
Bateman, W. and Mendel, G. (1909) Mendel’s Principles of Heredity. pp. 1–450.
Boyle, E.A. et al. (2017) An expanded view of complex traits: from polygenic to omnigenic. Cell, 169, 1177–1186.
Brockmann, G.A. et al. (2000) Single QTL effects, epistasis, and pleiotropy account for two-thirds of the phenotypic F2 variance of growth and obesity in DU61 x DBA/2 mice. Genome Res., 10, 1941–1957.

Calvin, C.M. et al. (2012) Multivariate genetic analyses of cognition and academic achievement from two population samples of 174,000 and 166,000 school children. Behav. Genet., 42, 699–710.

Chen, C.C.M. et al. (2011) Methods for identifying SNP interactions: a review on variations of logic regression, Random Forest and Bayesian logistic regression. IEEE/ACM Trans. Comput. Biol. Bioinf., 8, 1580–1591.

Cheng, Y. et al. (2011) Mapping genetic loci that interact with myostatin to affect growth traits. Heredity (Edinb), 107, 565–573.

Fisher, R.A. (1919) XV.—The correlation between relatives on the supposition of Mendelian Inheritance. Trans. R. Soc. Edinburgh, 52, 399–433.

García-Magariños, M. et al. (2009) Evaluating the ability of tree-based methods and logistic regression for the detection of SNP-SNP interaction. Ann. Hum. Genet., 73, 360–369.

Grice, S.J. et al. (2015) Synergistic interactions between Drosophila orthologues of genes spanned by de novo human CNVs support multiple-hit models of Mendelian Inheritance. Genes Brain Behav., 15, 280–290.

Hill, W.G. et al. (2008) Data and theory point to mainly additive genetic variance for complex traits. PLoS Genet., 4, e1000008.

Huang, W. et al. (2012) Epistasis dominates the genetic architecture of Drosophila quantitative traits. Proc. Natl. Acad. Sci. USA, 109, 15553–15559.

Huang, W. and Mackay, T.F.C. (2016) The genetic architecture of quantitative traits cannot be inferred from variance component analysis. PLoS Genet., 12, e1006421.

Jang, K.L. et al. (1996) Heritability of the big five personality dimensions and their facets: a Twin Study. Proc. Natl. Acad. Sci. USA, 93, 15273–15278.

Kuhn, M. and Johnson, K. (eds) (2013) Introduction. In: Applied Predictive Modeling. Springer, New York, NY, pp. 1–16.

Loo, J. et al. (2003) The behavior genetics of personality and the NEAD study. J. Res. Pers., 37, 373–387.

Luciano, M. et al. (2013) A genome-wide association study for reading and language abilities in two population cohorts. Genes Brain Behav., 12, 645–652.

Mackay, T.F. and Moore, J.H. (2014) Why epistasis is important for tackling complex human disease genetics. Genome Med., 6, 125.

Mackay, T.F.C. (2014) Epistasis and quantitative traits: using model organisms to study gene–gene interactions. Nat. Rev. Genet., 15, 22–33.

Michaelson, J.J. et al. (2017) Neuronal PAS domain proteins 1 and 3 are master regulators of neuropsychiatric risk genes. Biol. Psychiatry, 82, 213–223.

de Moor, M.H.M. et al. (2012) Meta-analysis of genome-wide association studies for personality. Mol. Psychiatry, 17, 337–349.

Nicodemus, K.K. et al. (2014) Variability in working memory performance explained by epistasis vs polygenic scores in the ZNF804A pathway. JAMA Psychiatry, 71, 778–785.

Okbay, A. et al. (2016) Genome-wide association study identifies 74 loci associated with educational attainment. Nature, 533, 539–542.

Power, K.A. and Pluess, M. (2015) Heritability estimates of the Big Five personality traits based on common genetic variants. Transl. Psychiatry, 5, e604.

Purcell, S. et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet., 81, 559–575.

Quinlan, J.R. (1992) C4.5: Programs for Machine Learning. Morgan Kaufmann, San Mateo, California.

Rietsveld, C.A. et al. (2013) GWAS of 126,559 individuals identifies genetic variants associated with educational attainment. Science, 340, 1467–1471.

Ruczinski, I. et al. (2004) Exploring interactions in high-dimensional genomic data: an overview of Logic Regression, with applications. J. Multivar. Anal., 90, 178–195.

Ruczinski, I. et al. (2003) Logic Regression. J. Comput. Graph. Stat., 12, 473–511.

Sackton, T.B. and Harrell, D.L. (2016) Genotypic context and epistasis in individuals and populations. Cell, 166, 279–287.

Schwender, H. and Ickstadt, K. (2008) Identification of SNP interactions using logistic regression. Bioinformatics, 9, 187–198.

Smith, B.H. et al. (2013) Cohort profile: generation scotland: scottish family health study (GS: sFH). The study, its participants and their potential for genetic research on health and illness. Int. J. Epidemiol., 42, 689–700.

Smith, B.H. et al. (2006) Generation Scotland: the Scottish Family Health Study; a new resource for researching genes and heritability. BMC Med. Genet., 7, 74.

Speed, D. et al. (2012) Improved heritability estimation from genome-wide SNPs. Am. J. Hum. Genet., 91, 1011–1021.

Stephan, J. et al. (2015) A random forest approach to capture genetic effects in the presence of population structure. Nat. Commun., 6.

Vukasović, T. and Bratko, D. (2015) Heritability of personality: a meta-analysis of behavior genetic studies. Psychol. Bull., 141, 769–785.

Webber, C. (2017) Epistasis in neuropsychiatric disorders. Trends Genet., 33, 256–265.

Wu, X. et al. (2008) Top 10 algorithms in data mining. Knowl. Inf. Syst., 14, 1–37.

Yang, J. et al. (2011) GCTA: a tool for genome-wide complex trait analysis. Am. J. Hum. Genet., 88, 76–82.

Zhao, Y. et al. (2012) Correction for population stratification in random forest analysis. Int. J. Epidemiol., 41, 1798–1806.