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A Family-Based Association Test for Repeatedly Measured Quantitative Traits Adjusting for Unknown Environmental and/or Polygenic Effects

Christoph Lange, *Department of Biostatistics, Harvard School of Public Health, 655 Huntington Avenue, Boston, MA 02115, USA*
Kristel van Steen, *Department of Biostatistics, Harvard School of Public Health, 655 Huntington Ave, Boston, MA 02115*
Toby Andrew, *Twin Research & Genetic Epidemiology Unit, St Thomas' Hospital, London SE1 7EH, UK*
Helen Lyon, *Brigham and Women's Hospital, Harvard Medical School, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115, USA*
Dawn L. DeMeo, *Brigham and Women*
Benjamin Raby, *Brigham and Women's Hospital, Harvard Medical School, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115, USA*
Amy Murphy, *Department of Biostatistics, Harvard School of Public Health, 655 Huntington Avenue, Boston, MA 02115, USA*
Edwin K. Silverman, *Brigham and Women*
Alex MacGregor , *Twin Research & Genetic Epidemiology Unit, St Thomas' Hospital, London SE1 7EH, UK*
Scott T. Weiss, *Brigham and Women's Hospital, Harvard Medical School, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115, USA*
Nan M. Laird, *Department of Biostatistics, Harvard School of Public Health, 655 Huntington Avenue, Boston, MA 02115, USA*

A Family-Based Association Test for Repeatedly Measured Quantitative Traits Adjusting for Unknown Environmental and/or Polygenic Effects*

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Abstract

We propose a family-based association test, FBAT-PC, for studies with quantitative traits that are measured repeatedly. The traits may be influenced by partially or completely unknown factors that may vary for each measurement. Using generalized principal component analysis, FBAT-PC amplifies the genetic effects of each measurement by constructing an overall phenotype with maximal heritability. Analytically, and in the simulation studies, we compare FBAT-PC with standard methodology and assess both the heritability of the overall phenotype and the power of FBAT-PC. Compared to univariate analysis, FBAT-PC achieves power gains of up to 200%. Applications of FBAT-PC to an osteoporosis study and to an asthma study show the practical relevance of FBAT-PC. FBAT-PC has been implemented in the software package PBAT and is freely available at http://www.biostat.harvard.edu/∼clange/default.htm.

KEYWORDS: family-based association test, unknown environmental and/or polygenic effects

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1 Introduction

In many studies of complex diseases, repeated measurements of disease related traits are available. For such studies, we propose a family based association test, FBAT-PC, that uses these repeated measurements. For example in osteoporosis studies, bone mineral density measurements are important underlying traits. They describe the potential severity of the disease and are usually measured at different body site locations in such as the spine and the hip. Their measurements depend upon the site location and other factors such as age, and weight and degree of exercise. In other studies repeated measures may be taken over time.

A major feature of a quantitative traits is that they are often influenced by other factors which are not of direct interest. For example in asthma, the measurements of forced expiratory lung volume measured before and after bronchodilator (PREFEV1 and POSFEV1) are important disease-related phenotypes. Nevertheless, their unadjusted, raw measurements do not reveal the affection status because the measurements of PREFEV1 and POSFEV1 depend also upon body characteristics, e.g. height, age, weight, sex, etc, and upon environmental factors, e.g. smoking, etc . As a result, such quantitative traits are ordinarily adjusted in a regression analysis. For FEV1-measurements, the variables height, age, weight and sex are known to be important predictor variables and adjustment formulas based on external standards have been derived (Weiss and Ware (1996)). Since these formulas are based on data from unaffected individuals and do not include other important covariates, e.g. smoking, their applicability to a diseased population is debatable. An alternative approach to using established adjustment formulas is to regress the traits on the covariates measured in the study. This requires careful model building and knowledge about the effects of the covariates. Theoretically, all environmental factors and covariates have to be known and measured. Either way, both internal and external adjustment approaches are prone to potential errors and mis-specifications, and the p-values for the association tests will depend upon the selected adjustment variables. This makes the interpretation of the test results difficult and raises questions about its reproducibility in other studies, especially when the other studies do not have data on some of the variables that were used in the adjustment of the significant finding.

The FBAT-PC statistic is based on applying generalized principal component analysis to both the phenotypic and the genetic variance matrix. It is similar to the generalized principal component approach for linkage analysis introduced by Rabinowitz and Ott (1998). When there are numerous traits, or repeated measures of traits, principal component analysis on the pheno-

typic variance matrix is commonly used as a variable reduction technique. Our approach differs in applying generalized principal component analysis to both the estimated genetic variance matrix and the phenotypic variance matrix. Using the conditional mean model approach introduced in Lange et al (2003b,c), we model the repeated measurements as a function of the imputed marker score and estimate the phenotypic and genetic variance for each measurement without biasing the significance level of the subsequently computed FBAT statistic. In the absence of proper adjustment, the genetic variance attributable to the marker will be low for any single repeated measure and a univariate test is unlikely to detect an association. Using generalized principal component analysis, FBAT-PC amplifies the small genetic effects for each measurement by constructing a single overall phenotype that has maximal heritability. We show that the overall FBAT-PC phenotype aggregates all the heritability contained in the quantitative measurements.

For complex disease mapping, the FBAT-PC statistic provides an alternative approach to regression-based methods for the analysis of disease-related quantitative traits that are influenced by other factors. Instead of trying to model the impact of other factors on the trait, FBAT-PC relies on additional measurements of the trait.

2 Methods

For sake of simplicity, we assume that trios (one offspring and the parents) are given, that the marker locus is the disease locus, and that the mode of inheritance is additive. All of these assumptions can be relaxed straightforwardly. Using the sufficient statistic by Rabinowitz and Laird (2000) instead of the parental genotypes, the FBAT-PC methodology extends to designs where parental genotypes are missing and additional siblings are available. If haplotypes are analyzed, then the FBAT-PC approach can be extended by using the sufficient statistic in the haplotype approach by Horvath et al (2003). For a multi-allelic marker, the approach by Laird et al (2000) can be applied.

In the study, n independent trios are sampled and a bi-allelic marker locus with alleles A and B is genotyped. We denote the number of transmitted A alleles in the offspring of the ith family by X_i. For each offspring, the same quantitative trait is measured m-times. The m measurements are given by $y_{i1}, \ldots, y_{im}$ and the corresponding vector by $\mathbf{y}_i$. The $(n \times m)$-matrix containing all phenotypes is given by $\mathbf{y} = (y_{ij})$. The standard biometric model describing the measurements of the quantitative trait as a function of the genotype is

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given by (Falconer & Mackay (1997))

$$E\left(Y_{ij}\,|X_i = x_i\right) \;=\; \mu_j + a(x_i, j) \tag{2.1}$$

where μ_j is the overall-mean for the jth measurement and $a(.,.)$ a function that depends upon the marker score, the mode of inheritance and the index j. When covariates are known, they can be included in equation 2.1 (Lange et al (2003a,b)). If the specification of the mean model 2.1 is correct, including covariates will increase the power of the approach by reducing the residual variance. The advantages and disadvantages of including covariates in the mean model (2.1) will be illustrated in the data analysis section. Note that, provided the covariates are independent of x_i, omitting covariates will not bias the specification of the genetic effect.

For the ith offspring, the phenotypic variance matrix is defined by

$$Var\left((Y_{i1}, \ldots, Y_{im})\,|X_i = x_i\right) \;=\; \mathbf{V}_P \tag{2.2}$$

where $\mathbf{V}_P$ is an user-defined $(m \times m)$-dimensional variance matrix which may depend upon additional parameters, but does not depend upon on x_i. In practise, to avoid a mis-specification of the correlation structure of $\mathbf{V}_P$, the matrix $\mathbf{V}_P$ can always be selected to be unstructured, i.e. all off-diagonal elements of the corresponding correlation matrix are different. We assume that the variance includes the effects of all variables that are not modelled as covariates in equation 2.1, but have an influence on the measurements, e.g. unknown environmental factors, polygenic effects and other covariates which are difficult to assess and to model. We will use the term environmental correlation in describing the parameters in $\mathbf{V}_P$ even though it more broadly includes any factor unrelated to x_i. When all m measurements of the quantitative trait are made under exactly the same conditions, a simple alternative hypothesis is given by

$$H_a:\; a(x, j) \;=\; \alpha x, \tag{2.3}$$

where α is the constant genetic effect size for all measurements. The variable x denotes an individual's marker score, but we will subpress the subscript i for simplicity.

A constant genetic effect size for each measurement is not plausible when the measurements are taken over time and important factors are changing, e.g. FEV1 measurements for asthmatic children recorded in a clinical trial over several years, or when the measurements are taken at different locations in

the body, e.g. bone density measurements at the spine and hip in osteoporosis studies. For such scenarios, the alternative hypothesis is given by

$$H_a : \; a(x, j) = \alpha_j x \tag{2.4}$$

where α_j is the genetic effect size for the jth measurement. Although the underlying genetic effect on the quantitative trait might be constant, the observed genetic effect size α_j may differ for each measurement due to varying influences from other variables which are not adjusted for. Function (2.4) defines the general alternative hypothesis for the additive genetic model. It does not require any structural assumptions about the dependence of the genetic size on the conditions for the jth measurement. At each measurement, the quantitative trait can be influenced by varying factors that are unknown or difficult to model.

Assuming for now that all parameters in the model defined by (2.1), (2.2) and (2.4) are known, the genetic variance matrix $\mathbf{V}_G$ under model (2.4) is computed as

$$\mathbf{V}_G \;=\; Var(\alpha_1 x, \ldots, \alpha_m x) \;\;=\;\; \Lambda \mathbf{1}\mathbf{1}^T \Lambda \tag{2.5}$$

where $\mathbf{1}$ is a m-dimensional vector of signs defined by $\mathbf{1} = (sign(\alpha_1), \ldots, sign(\alpha_m))^T$, and Λ is a diagonal matrix given by the square roots of the genetic variances for each measurement, i.e. $\Lambda = diag(\sqrt{Var(a_1 X)}, \ldots, \sqrt{Var(a_m X)})$. The diagonal matrix Λ depends upon the mode of inheritance, the allele frequencies and the genetic effect sizes α_j. The genetic variance matrix $\mathbf{V}_G$ defined by (2.5) has rank 1. Its construction relies on the assumptions that the same marker is analyzed for each trait and that the mode of inheritance is the same for all traits.

The goal of the generalized principal component analysis is to find a weight vector $\mathbf{w}_1, \ldots, \mathbf{w}_m$ that maximizes the relative genetic effect size $h^2(\mathbf{w}_j)$ of the overall phenotype $\mathbf{y}\mathbf{w}_j$, i.e. maximizing the heritability attributable to the SNP of interest (Ott and Rabinowitz (1999)),

$$h^2(\mathbf{w}_j) \;\;=\;\; \frac{\mathbf{w}_j^t \mathbf{V}_G \mathbf{w}_j}{\mathbf{w}_j^t \left(\mathbf{V}_G + \mathbf{V}_P\right) \mathbf{w}_j}. \tag{2.6}$$

Note that the total phenotypic variation is $\mathbf{V}_T = \mathbf{V}_P + \mathbf{V}_G$. The solution of this maximization problem is obtained by solving the generalized eigensystem (Press et al (1991))

$$\mathbf{V}_G \, \mathbf{w} \;\;=\;\; \lambda \mathbf{V}_P \, \mathbf{w} \tag{2.7}$$

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for the eigenvector $\mathbf{w}$. The vector $\mathbf{w}$ is derived in two steps. First we compute the Cholesky decomposition of $\mathbf{V}_P = \mathbf{L}\mathbf{L}^t$ where $\mathbf{L}$ is a lower triangular matrix. Then the eigenvalue decomposition for the eigensystem

$$\mathbf{L}^{-}\mathbf{V}_G(\mathbf{L}^t)^{-}\tilde{\mathbf{w}} = \tilde{\lambda}\tilde{\mathbf{w}} \tag{2.8}$$

is solved. The eigenvalues of system (2.8) are also eigenvalues of system (2.7). The eigenvectors of the generalized eigensystem (2.7) can be obtained by multiplying the eigenvectors of system (2.8) with $(L^t)^{-}$.

Since the correlation matrix $\mathbf{1}\mathbf{1}^t$ in equation (2.5) has rank 1, eigensystem (2.8) has only one non-zero eigenvalue. We denote this eigenvalue by $\tilde{\lambda}_h$ and the corresponding eigenvector by $\tilde{\mathbf{w}}_h$. The generalized eigensytem (2.7) has therefore also only one non-zero eigenvalue $\tilde{\lambda}_h$ with corresponding eigenvector

$$\mathbf{w}_h \;=\; (L^t)^{-}\tilde{\mathbf{w}}_h. \tag{2.9}$$

All remaining eigenvalues of system (2.7) are zero. Thus, the first generalized principal component, $\mathbf{y}\mathbf{w}_h$, combines all the genetic components of the m measurements of the quantitative trait. The remaining generalized principal components have zero heritability. For the ith offspring, the overall phenotype amplifying the heritabilities of all measurements is given by

$$y_i^{PC} \;=\; \mathbf{y}_i^t\mathbf{w}_h. \tag{2.10}$$

In conclusion, when a quantitative trait is measured several times under varying conditions, an overall phenotype can be constructed which amplifies the trait heritability by aggregating all the genetic components of all measurements into a single univariate phenotype with maximal heritability. The overall phenotype y_i^{PC} is then treated as a univariate quantitative trait and is tested for association, using the univariate quantitative FBAT statistic (Laird et al (2000), Lange et al (2002)). In this paper, the FBAT statistic applied to the overall phenotype $y_1^{PC}, \ldots, y_n^{PC}$ is referred to as *FBAT-PC*.

Unless we know the genetic variance matrix $\mathbf{V}_G$ and the phenotypic variance matrix $\mathbf{V}_P$, we can not compute FBAT-PC. We therefore need to estimate all parameters in (2.1), (2.2) and (2.5). Since the estimates have to be obtained without biasing the significance level of any subsequently computed FBAT statistics, we apply the conditional mean model approach by Lange et al (2003b,c). In the mean equation (2.1), we replace the observed marker score x_i by the expected marker score $E(X_i\,|P_{1i}, P_{2i}\,)$, i.e.

$$E\,(Y_{ij}) \;=\; \mu_j + \alpha_j E(X_i\,|P_{1i}, P_{2i}\,), \tag{2.11}$$

where P_{1i} and P_{2i} denote parental genotypes.

Assuming the phenotypic mean is given by (2.11) and the phenotypic variance by (2.2), we select an appropriate correlation structure for $\mathbf{V}_p$ and estimate all parameters in the mean and variance assumption by the generalized estimating equation (GEE) approach (Liang & Zeger (1986)).

Analogous to the screening techniques proposed by Lange et al (2003b,c), the conditional mean model can also be used to find the subset of measurements of the quantitative trait for which FBAT-PC has maximal power. For each subset of measurements, we construct the overall FBAT-PC phenotype and estimate its heritability, using the GEE-estimates of the corresponding mean model. Applying the approach for conditional power calculations by Lange and Laird (2002) to the overall FBAT-PC phenotype, the power of FBAT-PC is computed for each subset of measurements without biasing the significance level of any subsequently computed FBAT-PC statistic. Instead of all measurements, the subset of measurements with the the maximal power could be tested. In the simulation study, we will compare this strategy with using all measurements for the construction of the overall FBAT-PC phenotype.

A further advantage of the conditional mean model approach is that the observed data can be used to infer the function $a(.,.)$ and the alternative hypothesis H_A. Since the conditional mean model (2.11) can be estimated as many times as needed without biasing the significance level of FBAT-PC, classical model building techniques can be applied to model (2.11) for the selection of both covariates and the appropriate function for $a(.,.)$. In this way, the data set can be used to infer the alternative hypothesis before computing the test statistic. Nevertheless, it is important to note that, for the computation of FBAT-PC, a specific alternative hypothesis is not necessary. Without specifying a particular alternative hypothesis, both variance matrices $\mathbf{V}_G$ and $\mathbf{V}_P$ can always be computed based upon the general alternative hypothesis (2.4). Since this approach requires the fewest assumptions inferred from the conditional mean model, it will be used throughout the paper.

FBAT-PC can also be constructed when measurements of the trait are missing. Then, for the parameter estimation, only the observed measurements are included in the conditional mean model. Using the estimated genetic variance matrix and the phenotypic variance matrix, $\mathbf{w}_h$ is computed for the observed measurements of each proband separately. Furthermore, it is important to note that the FBAT-PC approach can also be applied to multivariate phenotypes.

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3 Heritability considerations

In this section, we assess analytically the increase in the heritability of the overall phenotype constructed by FBAT-PC relative to a "standard" principal component analysis which does not use the marker data to determine the components. Discussing the heritability instead of the power has the advantage that our considerations are disentangled from the effects of effect size estimation, ascertainment conditions and population parameters, e.g. allele frequency and number of informative families. Without being influenced by the these effects, the dependence of the heritability of the overall FBAT-PC phenotype upon trait heritability and environmental correlation can be assessed directly. We compare the heritability of the overall FBAT-PC phenotype with the original trait heritability and the heritabilities of the overall phenotypes obtained by standard principal component analysis, i.e. maximizing the variance of the overall phenotype $Var\left(\mathbf{w}^t\mathbf{Y}_i\right) = \mathbf{w}^t\left(\mathbf{V}_G + \mathbf{V}_P\right)\mathbf{w}$. In standard principal component analysis for longitudinal data, the first component is usually a weighted mean of all measurements. The second principal component can often be interpreted as a slope. In our heritability considerations, we denote the first component of the standard principal component analysis by $\mathbf{y}_{pc}^{(1)}$ and the second component by $\mathbf{y}_{pc}^{(2)}$.

We assume that we observe the same quantitative trait at two time points, $j = 1$ and $j = 2$, and that the genetic effect size can be different for both measurements. The mean model is defined by (2.11), (2.1) and (2.4) with $\mu_j = 0$. The parameter α_j is the size of the additive gene effect at time point j. The overall genetic effect for $j = 1$ is given by h. The genetic effect size at time point $j = 2$ is measured by the proportion λ of the original heritability h. We will look at three scenarios:

- Scenario I ($\lambda = 1$): The quantitative trait is measured twice under exactly the same conditions and heritability is the same at both time points.

- Scenario II ($\lambda = 1/2$): The quantitative trait is measured twice under varying conditions. The heritability for the first measurement is h and $h/2$ for the second measurements.

- Scenario III ($\lambda = 0$): The quantitative trait is measured twice under varying conditions. The heritability for the first measurement is h and 0 for the second measurements.

We assume that the phenotypic variance matrix is given by

$$\mathbf{V}_P = \begin{pmatrix} 1 & r \\ r & 1 \end{pmatrix} \tag{3.1}$$

where r denotes the environmental correlation. The genetic variance matrix can be computed based on the heritability h, the parameter λ and the allele frequency p.

We compute analytically the heritability of the overall phenotype constructed by FBAT-PC, $h_{y^{(PC)}}$, and the heritabilities of the first two components of standard principal component analysis, $h_{\mathbf{y}_{PC}^{(1)}}$ and $h_{\mathbf{y}_{PC}^{(2)}}$. To compare FBAT-PC with the other approaches, we compute the relative difference between the approaches, i.e. $h_{y^{(PC)}}/h_{\mathbf{y}_{PC}^{(1)}} - 1$ and $h_{y^{(PC)}}/h_{\mathbf{y}_{PC}^{(2)}} - 1$. We also compare the heritability of the overall FBAT-PC phenotype with the measurement that has the highest heritability (say h), i.e. the heritability for time point $j = 1$, $h_{y^{(PC)}}/h - 1$. These heritability ratios do not depend upon the allele frequency p, although the initial expression for the genetic variance did depend upon the allele frequency p.

For scenario I ($\lambda = 1$), the analytical formulas are simple. The matrices $\mathbf{V}_P$ and $\mathbf{V}_G$ share the same eigenvectors, $(1,1)^T$ and $(1,-1)^T$, and $FBAT$ for $y_{PC}^{(1)}$ is equal to the overall phenotype constructed by FBAT-PC. The heritability of the second standard principal component $y_{PC}^{(2)}$ is zero. Thus the heritability ratios for $y_{PC}^{(1)}$ and $y_{PC}^{(2)}$ are 1 and ∞, respectively. The heritability ratio for FBAT-PC and Y_1 is $\frac{-2h}{-h+rh-r-1}$. This ratio minus 1 is plotted in Figure 1, which shows that the largest gains for FBAT-PC occur for low heritability and low environmental correlation.

For Scenario II ($\lambda = 1/2$) and Scenario III ($\lambda = 0$), the relative increases in heritability for the FBAT-PC phenotype are shown in Figure 3(a) -3(f). These figures show that, when the heritability varies for each observations, the gains for FBAT-PC can be large for low heritabilities and high environmental correlation. The most important plots are Figure 3(a) and 3(c). They compare the heritability of the FBAT-PC phenotype with the heritability of the univariate measurement for time point j=1, for which the heritability is maximal. When the environmental correlation is high and the heritability low, the original trait heritability can be amplified by several magnitudes using FBAT-PC, e.g. Figure 3(a) shows potential gains up to 400% over the original trait heritability. It is important to note that the heritability for FBAT-PC is greater for the scenarios in which the heritabilities varies for each observation (Scenario II and III) than for the scenario with constant heritability.

For all three scenarios, the FBAT-PC phenotype has the highest heritabil-

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ity. When the environmental correlation is high and the heritability of the quantitative trait is low, the gain in heritability by using the FBAT-PC phenotype is substantial and potentially of practical importance. The unadjusted raw measurements of a quantitative trait which are influenced by varying, but similar environmental factors for each measurement are expected to have both high environmental correlation and very low heritabilities. The large increase in heritability of the FBAT-PC phenotype under these circumstances suggest that FBAT-PC is a well suited test statistic for such repeated measurements. The increase in heritability will be further amplified by adding more measurements of the quantitative trait.

4 Simulation studies

In this section, we assess the power of FBAT-PC by simulation studies. The objective is to examine whether the advantageous heritability of the overall FBAT-PC phenotype translates into power increases of similar proportions, when the variance matrices $\mathbf{V}_G$ and $\mathbf{V}_P$ have to be estimated under the general alternative-hypothesis (2.4). We compare FBAT-PC with the multivariate FBAT statistic, FBAT-GEE (Lange et al (2002)), and with univariate FBATs applied to the first and second component of standard principal component analysis. Initially, we also applied FBAT-GEE just to the first two components of standard principal component analysis. Since this FBAT-GEE never outperformed the univariate FBAT for the first principal component, we omit it here.

We will examine the dependence of the power of each test statistic upon the allele frequency, varying heritabilities for each measurements and the number of measurements used in the test statistic.

The simulation study is designed around the osteoporosis study discussed in the data analysis section of this paper. The marker of interest is a bi-allelic locus. We generate the parental genotypes p_1 and p_2 by drawing from a binomial distribution $B(2,p)$ where p is the allele frequency of the target allele in the population. The genotype x_i of the proband is obtained by simulated Mendelian transmissions of the parental genotypes p_1 and p_2. For the computation of the FBAT statistics, the genotypes of probands and their parents are assumed to be known. For each proband, the same quantitative phenotype is measured 10-times. The 10-dimensional phenotypic vector $\mathbf{Y}_i$ for the ith offspring is a random sample from a multivariate normal distribution, i.e.$\mathbf{Y}_i \sim N\left((\alpha_1 x_i, \ldots, \alpha_{10} x_i), \mathbf{V}\right)$, where α_j is the additive genetic effect for the jth measurement. The phenotypic variance matrix $\mathbf{V}_p$ is selected to be the

phenotypic correlation matrix of the 10 bone-density measurements in the osteoporosis study. The strength of the additive effect relative to the phenotypic variance is measured by the heritability h^2.

First, we conducted the simulation study for constant genetic effect size across all 10 measurements (constant heritability h). In each replicate of the simulation study, we generate $n = 200$ trios. Each offspring has 10 repeated measurements that are simulated from a multivariate normal distribution with constant effect sizes $\alpha_1 = \alpha_2 = \ldots = \alpha_{10}$. α_1 is computed from the elected heritability h. The overall-significance level is set to be 1%. The 10 phenotypes are tested for association, using FBAT-PC, FBAT-GEE and univariate quantitative FBATs for the first and second component of standard principal component analysis. To examine the effect of the number of measurements on the power of all four tests, we apply the four tests subsequently to the first k measurements of the trait, $k = 1, .., 10$.

The simulation study is repeated 100,000 times for a variety of different allele frequencies. Since the results for all allele frequencies are very similar, we report only the results for allele frequencies $p = 0.05$ and 0.10. The heritabilities used in this simulation study are 0.01, 0.025, 0.05 and 0.10. These values are relatively small compared to other simulation studies published in the literature, especially given the sample size of only 200 trios. The very small heritabilities are meant to reflect the idea that the raw, unadjusted measurements of a continuous trait are used in the test statistics.

The simulation study is then repeated under the assumption of varying genetic effect sizes for each measurement (varying h). In this simulation study, the additive effect for the jth measurement is given by

$$a_j \sim U_j(0, 2\alpha_h), \tag{4.1}$$

where U_j is a random variable that is uniformly distributed on the interval $[0, 2\alpha_h]$. The variable α_h is the additive effect size that corresponds to the given heritability h.

The power of each test was estimated by the proportion of the number of times the test statistic was significant at the 1% level. The plots of the power estimates for all 4 FBATs and both scenarios (constant heritability and varying heritability) are shown in Figures 3 and 4. Figures 3 and 4 also contain the plots for the estimated significance levels for each test. The significance levels were estimated in the same way as the power. Under the null-hypothesis, we generated the phenotypes from a multivariate normal distribution with no genetic effect size, i.e. $\mathbf{Y}_i \sim N\left((\alpha_1 x_i, \ldots, \alpha_{10} x_i), \mathbf{V}\right), \alpha_1 = \alpha_2 = \ldots = \alpha_{10} = 0.0$. Figure 3 and 4 show that the significance level is well preserved. Given

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the theoretical derivation of the FBAT-PC statistic as well as the discussion of the conditional mean model in Lange et al (2003b,c), this observation has been expected.

For both scenarios, it is important to note that the curves of the power estimates for all 4 test statistics originate from the same point for $k = 1$. For $k = 1$, all 4 test statistics simplify to the same univariate quantitative FBAT statistic. The estimated power level for $k = 1$ is a reference point to examine whether it is worthwhile to use repeated measurements instead of a single measurement.

When all measurements are taken under the same conditions (constant heritability), the FBAT statistic using the first component of the standard principal component analysis performs best, followed by the FBAT-PC approach. If the genetic variance matrix is known to be constant for all measurements and assumed so in the estimator of $\mathbf{V}_G$, both approaches, FBAT-PC and FBAT using the first component of the standard principal component analysis, are identical. The power differences between these two approaches in the "constant h"-case are therefore attributable to the uncertainty in the specification of the alternative hypothesis in the FBAT-PC statistic, using alternative hypothesis (2.4). The power levels achieved by FBAT-GEE are low for this scenario.

With varying h, FBAT-PC outperforms all the other approaches. FBAT-GEE, which does not make any distributional assumptions for the measurements, achieves power level similar to FBAT-PC, if the number of measurements is relatively small ($k < 5$). While for larger numbers of measurements the power estimates of FBAT-PC continue to increase, the power estimates for FBAT-GEE are reduced.

A pattern that is common to all power curves (Figure 3 and 4) is the change of the curves at $k = 4$. For the case of constant heritability, Figure 3 and 4 show power curves that, for $k \leq 4$, do not increase much, and, for $k > 4$, start to increase rapidly. The situation is reversed for the case of varying heritability. For $k \leq 4$, the gain in power is substantially, while, for $k > 4$, the formations of plateaus is observed. These different behaviors of the curves can be explained by looking at both the heritability considerations of the previous section and the structure of the phenotypic correlation matrix used in the simulation study. Figure 1 shows the plots of the correlation values between the first k measurements for $k = 2, \ldots, 10$. For $k \leq 4$, all correlation values are in the range between 0.8 and 1.0. For $k > 4$, measurements are added that are not as highly correlated (≈ 0.4) as the first 4 measurements. For the case of constant heritability, the analytical heritability calculations for Figure 1 explain this observation. While almost no heritability is gained by adding highly correlated measurements to the FBAT-PC phenotype, a considerable

increase in heritability for the FBAT-PC phenotype can be accomplished by including measurements with smaller environmental correlation in the overall phenotype. For the case of varying heritability, the rapid increases of the power curves for ($k \leq 4$) reflect the large gains in heritability for highly correlated measurements (Figure 3(a) and 3(c)). For moderate heritabilities, the gains in Figure 3(a) and 3(c) become small which explains the formation of plateaus.

In all cases (constant and varying heritability) and for all parameters (allele frequency, heritability, ...), FBAT-PC and FBAT based on the first component of standard principal component analysis are most advantageous. The simulation studies suggest that such "FBAT-PC"s can be powerful tools in situations in which the researcher has recorded repeated measurements of a quantitative trait whose genetic component is diluted by other known/unknown variables. When all measurements are taken under the assumption that the observable genetic effect is constant for all measurements, it seems to be most favorable to construct an overall phenotype based on standard principal component analysis and test it with the univariate FBAT statistic.

However, for the more realistic scenario, when the conditions under which the measurements are recorded vary and may have an influence on the observable genetic effect, FBAT-PC appears to be most powerful. Further, comparing the estimated power for both cases (constant and varying heritability), higher power levels are achieved when the measurements are taken under conditions with varying observable genetic effect sizes than for constant genetic effect sizes. The heritability calculations of the previous section agree with this observation (Figure 1, 3(a) and 3(c)). It is important to note that the power levels achieved by FBAT-PC can be up to 200% bigger than the power of the univariate FBAT testing one single observation.

For FBAT-PC, the results of our simulation study also show that all measurements should be incorporated in the overall-phenotypes to achieve maximal power. The power levels of FBAT-PC seems to increase monotonically with the number of observations.

Further, the plots of the estimated significance levels show that all test statistics maintain the specified significance level. We also repeated the simulation study, assuming population admixture and stratification (e.g. Lange et el (2003b,c)). In the presence of mild and moderate population admixture and stratification, the power of FBAT-PC is reduced slightly, but it still outperforms all the other methods substantially.

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5 Data analysis I: An osteoporosis candidate marker study in twins

In a previous confirmatory candidate marker study (Andrew et al 2002), we investigated possible linkage and association for bone mineral density (BMD), heel ultrasound and bone turnover with the osteocalcin gene (BGLAP) using a nearby (50-80kb) candidate microsatellite marker, D1S3737. Non-identical twin sisters aged 18-75 years at first interview were recruited for the study from the St Thomas' UK Adult Twin Registry with 1366 women being genotyped for the marker D1S3737. Linkage, allelic association and joint linkage and association tests were carried out.

For allele 10, we found BMD and ultrasound variables showed evidence of linkage and association with the marker in post menopausal women. The data set contained 630 postmenopausal women. For the other alleles, no significant association could be detected.

In this study we selected allele 4 of marker D1S3737. The allele frequency is 15.8%. We used bone mineral density variables measured at ten key different body sites: lumbar spine joints 1, 2 ,3 and 4, the left radius, the left ulna, the left hip neck, left hip total BMD, the left hip inter-trochanter site and the left hip wards triangle. All BMD measurements were made using dual-energy X-ray absorptiometry on a Hologic QDR-2000 (Hologic, Waltham, Massachusetts, USA). We tested the 4-allele for association with the 10 bone density measurements in post menopausal women, using FBAT-PC, FBAT-GEE and univariate FBATs applied to the first and second component of standard principal component analysis.

Since the bone density measurements were taken at different locations in the spine and in the hip of the patients, the heritabilities for each measurement are expected to differ. For such measurements, the previous simulation study suggests that FBAT-PC is the favorable FBAT statistic. We compare the results for FBAT-PC, with FBAT-GEE, univariate FBATs applied to the first and second component of standard principal component analysis and univariate FBAT's for each of the 10 measurements. All analyses used in offset as described in Laird et al (2000).

The data analysis is conducted twice. First, we adjusted the bone density measurements for age and body mass index by including these variables as covariates in the conditional mean equation (2.1) separately for each measurements. Then, the analysis was repeated without covariates. The results are given in Table 1. Further, Table 1 contains the results for the univariate quantitative FBATs testing each bone density measurement separately.

The only other association test for family studies that does not use the non-informative families is the PDT by Monks and Kaplan (2000). We therefore computed also all test statistics based on the PTD statistic rather than on the FBAT statistic. The results were very similar. Given the theoretical similarities between both approaches (Lange et al (2002)), these results were expected.

The estimates for the power of the FBAT-statistics in Table 1 are computed based on the effect sizes estimates obtained from the conditional mean model (2.1). The heritability estimates for the 10 BMD measurements are derived from the genetic effect size estimates using the conditional mean model (2.1). The heritability estimates vary considerably for all ten locations in the spine. Under these circumstances, i.e. varying genetic effect sizes, our power considerations suggested that FBAT-PC outperforms all other tests, including testing the first principal component with FBAT. This is also reflected in the power estimates in Table 1. While FBAT-PC achieves a power level of about 30%, all other tests have power levels of not more than 10%.

The advantages of FBAT-PC over the other tests in Table 1 are clear. FBAT-PC is the only test with the sufficient power to detect a significant association. Another important observation in Table 1 is the lack of effect of including covariates in the computation of FBAT-PC. The differences in p-values for both test statistics are not meaningful at all. Considering the danger of potential model mis-specification, Table 1 suggests that including covariates in FBAT-PC is not worthwhile.

6 Data Analysis II: An asthma study (CAMP)

We applied FBAT-PC to a collection of parent/child trios in the Childhood Asthma Management Program (CAMP) Genetics Ancillary Study. The CAMP study randomized asthmatic children to three different asthma treatments (CAMP 1999). Blood samples for DNA were collected from 696 complete parent/child trios from 640 nuclear families in the CAMP Ancillary Genetics Study. Genotyping was performed at 4 polymorphic loci located in the IL10 gene (Lyon et al (2003)). FEV1 measurements for pre and post bronchodilator were taken at 12 time points over 4 years. FEV1 is known to be an important asthma phenotype that also depends on the age, height, weight and sex of the individual. It is standard practice to adjust the FEV1 measurements for these covariates before analyzing them. One can adjust the FEV1 measurement using standard adjustment formulas for age, height, weight and sex, and obtain the so called "percent predicted" value (Weiss and Ware (1986)). Alternatively,

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using the conditional mean model approach (Lange et al (2003b,c)), one can regress the FEV1 measurements on the recorded values for age, height, weight and sex and use the residuals in the association analysis. We will compare both adjustment methods with using unadjusted, raw measurements.

First, we analyze all 12 FEV1 measurements (pre and post bronchodilator), using FBAT-PC. We apply FBAT-PC to the raw unadjusted measurements, the residuals obtained from the conditional mean model and to the mean-centered "percent predicted"-values (Weiss and Ware (1986)). All tests apply the FBAT offset to mean-centered traits (Laird et al (2000)). Table 2 shows the results of the FBAT-PC statistics for the FEV1-measurements before and after bronchodilator, respectively. Second, for marker $m1$, Table 3 contains the results of univariate FBAT analysis, testing each measurement separately.

Table 2 provides strong evidence for an association between all 4 SNPs in IL10 and the FEV1-measurements. When FBAT-PC is applied to the raw, unadjusted measurements, the results are completely consistent for pre and post bronchodilator. The p-values for the adjusted phenotypes are slightly less convincing. Using the "percent predicted" measurements, the test results lack consistency and fail to detect an association for marker $m3$ and $m6$.

The univariate FBAT-analysis for marker $m1$ (Table 3) shows a few barely significant results, but no convincing pattern of significance. However, the heritability estimates for the adjusted and the "percent predicted" measurements are on average higher than for the raw measurements.

The asthma study also illustrates the strength of FBAT-PC. We are able to establish strong significance between FEV1-measurements and a set of SNPs in IL10 that would have not been detected with other tests. The example demonstrate that, when repeated measurements with relatively small, varying genetic effect sizes are recorded, FBAT-PC can be used to combine all genetic effects into one new phenotype without having to adjust for other known/unknown covariates. The overall phenotype constructed by FBAT-GEE has a much higher heritability than the univariate tests and can therefore be tested with sufficient power.

7 Discussion

We propose a family-based association test, FBAT-PC, that is designed for situations in which repeated measurements of a quantitative trait have been recorded and the genetic effect in each measurement may be diluted by other known or unknown factors.

In this situation, one typically measures all variables that might influence

the quantitative trait and models their influence on the quantitative trait. The inconsistent results for the adjusted measurements of both the osteoporosis study and the asthma study illustrate that this can be a big challenge, even for relatively well-understood traits such as FEV1 measurements. On the other hand, FBAT-PC does not require any adjustment of the quantitative measurements. Instead of measuring potential covariates of the trait and using them to reduce residual variation, the quantitative trait of interest is measured several times. By extracting the genetic components of all measurements and combining them into one overall phenotype with maximal heritability, a powerful test statistic can be constructed. For many complex diseases, obtaining repeated measurements of the quantitative trait of interest can be less problematic than the potentially error-prone adjustment-approaches.

FBAT-PC has been implemented in PBAT (Lange et al (2004)) and is freely available at http://www.biostat.harvard.edu/ clange/default.htm.

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Table 1. Twin study on osteoporosis: FBAT results for allele 4 (57 informative families). Using the approach by Lange et al (2004b), the power is estimated for a significance level of $\alpha = 5\%$.

		with covariates			without covariates		
test statistic	BMD-measurements	p-value	power*	h	p-value	power*	h
FBAT-PC	all	0.002	0.360	0.0360	0.003	0.340	0.0320
$FBAT - PC^1$	all	0.858	0.055	0.0021	0.560	0.082	0.0040
$FBAT - PC^2$	all	0.935	0.069	0.0007	0.142	0.111	0.0016
FBAT-GEE	all	0.201	0.112	-	0.416	0.098	-
FBAT	lumbar spine joints 1 (BMD 1)	0.904	0.058	0.0006	0.519	0.067	0.0016
FBAT	lumbar spine joints 2 (BMD 2)	0.350	0.054	0.0005	0.575	0.057	0.0009
FBAT	lumbar spine joints 3 (BMD 3)	0.241	0.058	0.0008	0.428	0.051	0.0001
FBAT	lumbar spine joints 4 (BMD 4)	0.896	0.055	0.0005	0.544	0.062	0.0015
FBAT	the left radius (BMD 5)	0.886	0.061	0.0015	0.645	0.070	0.0030
FBAT	the left ulna (BMD 6)	0.733	0.067	0.0020	0.488	0.081	0.0040
FBAT	the left hip neck (BMD 7)	0.991	0.052	0.0002	0.723	0.056	0.0007
FBAT	left hip total BMD (BMD 8)	0.460	0.105	0.0049	0.274	0.119	0.0066
FBAT	the left hip inter-trochanter site (BMD 9)	0.627	0.077	0.0028	0.376	0.093	0.0048
FBAT	the left hip wards triangle (BMD 10)	0.622	0.097	0.0051	0.374	0.116	0.0079

Table 2. Childhood Asthma Management Program (CAMP): FBAT-PC results testing for association between the FEV1 measurements and SNPs in IL10. Using the approach by Lange et al (2004b), the power is estimated for a significance level of $\alpha = 5\%$.

	FBAT-PC for the PREFEV1-measurements								
	without covariates			with covariates			percent predicted		
SNP	p-value	power	h	p-value	power	h	p-value	power	h
m1	0.0007	0.67	0.12	0.0008	0.94	0.11	0.0022	0.63	0.13
m2	0.0178	0.58	0.08	0.0410	0.98	0.08	0.2284	0.80	0.09
m3	0.0011	0.57	0.11	0.0048	0.93	0.11	0.0016	0.52	0.12
m4	0.0134	0.62	0.09	0.0463	0.99	0.08	0.1831	0.86	0.09

	FBAT-PC for the POSTFEV1-measurements								
	without covariates			with covariates			percent predicted		
SNP	p-value	power	h	p-value	power	h	p-value	power	h
m1	0.0040	0.74	0.13	0.005	0.97	0.12	0.006	0.59	0.07
m2	0.0072	0.68	0.09	0.017	0.99	0.07	0.052	0.73	0.08
m3	0.0024	0.63	0.12	0.005	0.96	0.12	0.001	0.90	0.12
m4	0.0007	0.68	0.10	0.004	0.99	0.09	0.147	0.80	0.09

Table 3. Childhood Asthma Management Program (CAMP): Univariate FBAT results testing for association between the PREFEV1 measurements and SNP *m*1 in IL10. The power is estimated for a significance level of $\alpha = 5\%$.

	PREFEV1 measurements															
	without covariates			with covariates			percent predicted			without covariates			with covariates			percent predicted		
	p-value	pow.	h	p-value	pow.	h	p-value	pow.	h	p-value	pow.	h	p-value	pow.	h	p-value	pow.	h
0	0.6248	0.05	0.0392	0.5480	0.52	0.0471	0.4266	0.54	0.0435	0.4116	0.05	0.0392	0.4305	0.52	0.0718	0.2345	0.49	0.0356
1	0.0766	0.05	0.0368	0.6414	0.51	0.0471	0.0177	0.46	0.0346	0.3231	0.05	0.0368	0.6280	0.51	0.0566	0.0102	0.44	0.0336
2	0.6941	0.05	0.0313	0.6525	0.45	0.0461	0.2269	0.33	0.0237	0.7065	0.50	0.0313	0.6928	0.45	0.0538	0.1171	0.33	0.0238
3	0.3760	0.05	0.0405	0.5324	0.53	0.0617	0.0162	0.46	0.0383	0.5362	0.05	0.0405	0.5293	0.53	0.0702	0.0158	0.52	0.0412
4	0.7013	0.05	0.0225	0.7463	0.35	0.0406	0.0959	0.30	0.0202	0.8308	0.05	0.0225	0.9315	0.35	0.0496	0.1306	0.33	0.0234
5	0.6143	0.05	0.0184	0.3921	0.30	0.0321	0.7838	0.20	0.0118	0.5996	0.34	0.0184	0.2787	0.30	0.0385	0.9184	0.24	0.0138
6	0.9419	0.12	0.0067	0.5021	0.15	0.0099	0.5903	0.14	0.0065	0.8884	0.17	0.0067	0.7568	0.15	0.0181	0.4113	0.08	0.0081
7	0.7130	0.17	0.0057	0.1536	0.13	0.0131	0.1624	0.11	0.0044	0.8706	0.09	0.0057	0.2333	0.13	0.0070	0.1253	0.12	0.0052
8	0.4713	0.17	0.0074	0.0593	0.16	0.0108	0.1048	0.12	0.0052	0.7130	0.22	0.0074	0.1153	0.16	0.0193	0.0394	0.08	0.0034
9	0.2690	0.05	0.0001	0.4459	0.05	0.0000	0.1027	0.06	0.0003	0.4537	0.05	0.0001	0.3142	0.05	0.0087	0.0303	0.06	0.0007
10	0.6426	0.05	0.0004	0.2979	0.06	0.0012	0.4561	0.09	0.0021	0.7999	0.05	0.0004	0.1412	0.06	0.0000	0.8786	0.08	0.0017
11																		

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Fig. 1: Relative gain in heritability of *FBAT-PC* over univariate quantitative FBATs: Scenario I (constant heritability): $\frac{h_{FBAT-PC}}{h_y} - 1$

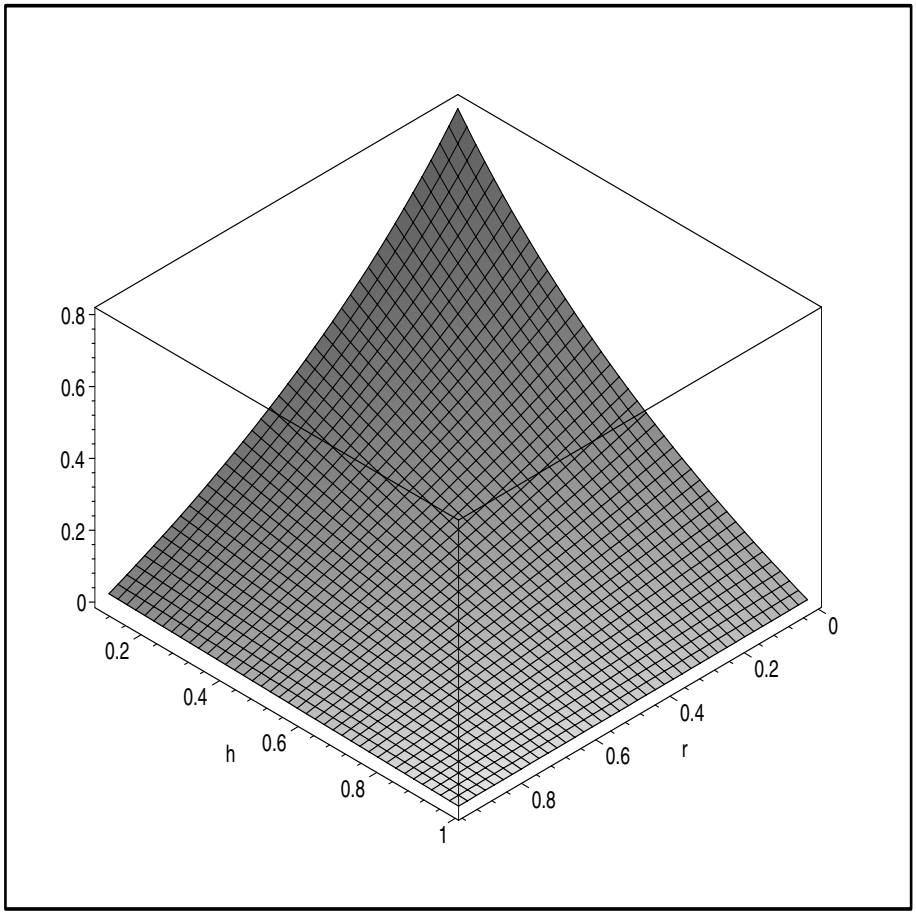
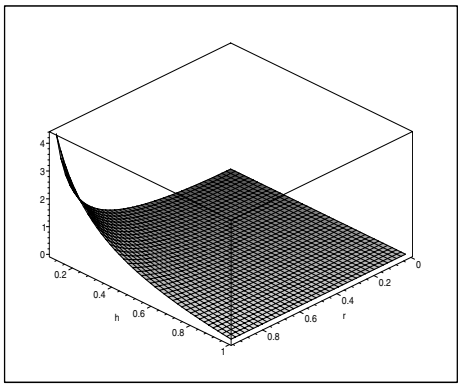
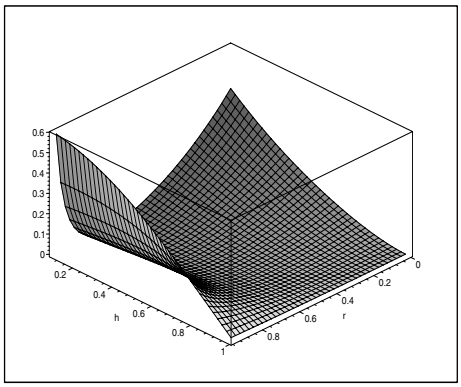


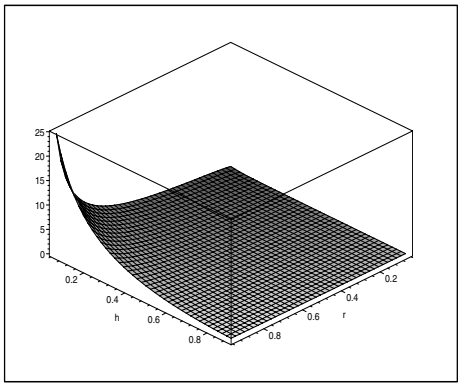
Fig. 2: Relative gain in heritability of *FBAT-PC* over univariate quantitative FBATs



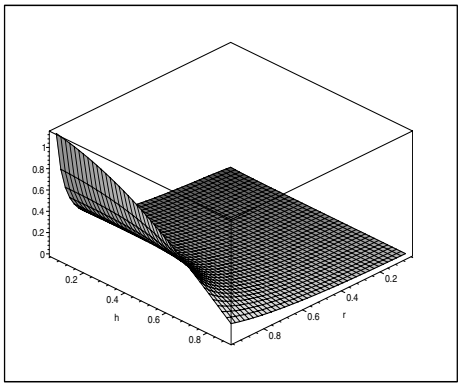
(a) Scenario II: $\frac{h_{FBAT-PC}}{h_y} - 1$



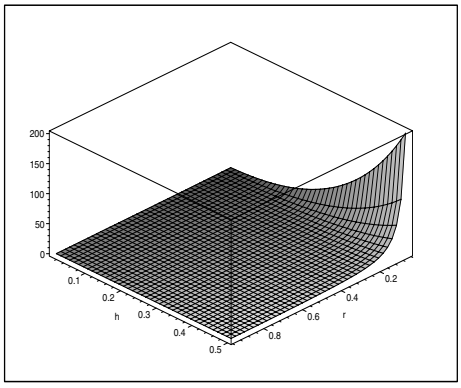
(b) Scenario III: $\frac{h_{FBAT-PC}}{h_y} - 1$



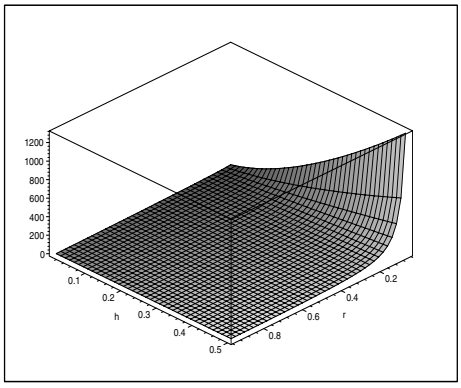
(c) Scenario II: $\frac{h_{FBAT-PC}}{h_{PC1}} - 1$



(d) Scenario III: $\frac{h_{FBAT-PC}}{h_{PC1}} - 1$



(e) Scenario II: $\frac{h_{FBAT-PC}}{h_{PC2}} - 1$



(f) Scenario III: $\frac{h_{FBAT-PC}}{h_{PC2}} - 1$

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Fig. 3: Estimated power levels for allele frequency $p = 0.05$. The plot for $h = 0.0$ shows the estimated significance levels for all four tests.

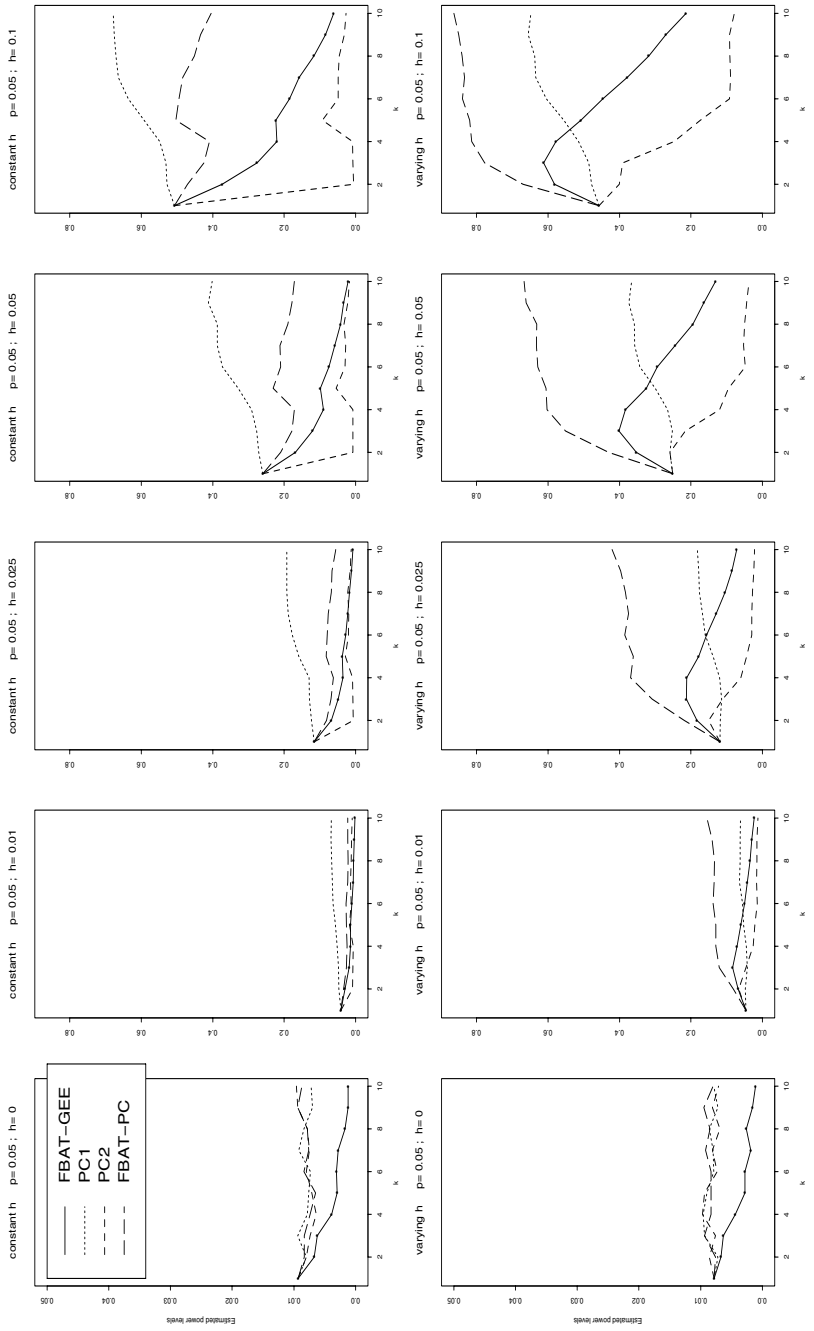

Fig. 4: Estimated power levels for allele frequency $p = 0.10$. The plot for $h = 0.0$ shows the estimated significance levels for all four tests.

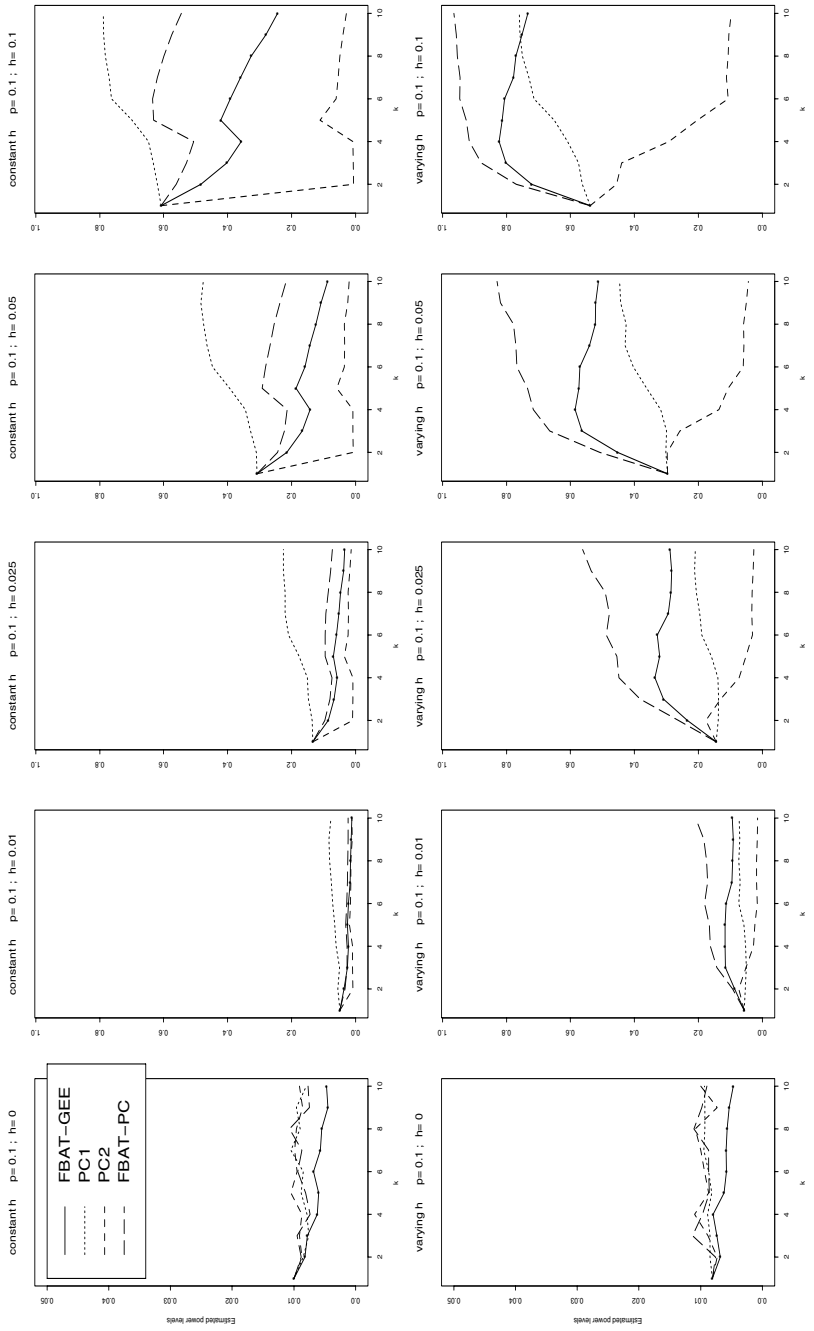

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Fig. 5: Phenotypic correlation of the bone density measurements for the first m measurments