Kafkas Univ Vet Fak Derg
23 (1): 87-94, 2017
DOI: 10.9775/kvfd.2016.15883

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Multiple Hypothesis Testing in a Genome Wide Association Study of Bovine Tuberculosis [1]

Burak KARACAÖREN [1]

[1] *Part of this study was presented as poster at XIVth Congress of Medical Biology and Genetics at Muğla/Turkey (27-30 October 2015) and published in congress abstract book*

[1] Department of Animal Science, Faculty of Agriculture, Akdeniz University, TR-07059 Antalya - TURKEY

Article Code: KVFD-2016-15883 Received: 14.04.2016 Accepted: 02.09.2016 Published Online: 09.09.2016

Citation of This Article

Karacaören B: Multiple hypothesis testing in a genome wide association study of bovine tuberculosis. *Kafkas Univ Vet Fak Derg,* 23, 87-94, 2017. DOI: 10.9775/kvfd.2016.15883

Abstract

Genome-wide association studies (GWAS) have been used to detect single nucleotide polymorphisms (SNPs) related to various animal traits. The outcome of GWAS is based on quality of the both phenotypic and genotypic datasets. False positive (or negative) associations can be obtained due to multiple hypothesis testing procedures, quality control measures, or an undetected population structure. The objectives of this study were to 1) investigate different multiple hypothesis testing procedures with different quality measures and 2) to detect and correct ancestral stratification using different single SNPs models of the bovine tuberculosis GWA data set. Based on a regression model, SNPs from chromosomes 2, 7, 8 and 13 were detected at a significance level of P<0.001 without correction for multiple hypothesis testing. However, after Bonferroni correction, Hochberg's method and permutation test for multiple hypothesis correction genomic signals, it became non-significant. Only a false discovery rate approach detected weak signals (at level of 0.54) from chromosomes 2, 8, and 13. We used a model that took into account the effect of linkage disequilibrium to the multiple hypothesis testing procedures by combining adjacent SNPs test statistics with windows sizes of 2, 4 and 6. We detected strong genomic signals from chromosomes 13, 8, 6 and 2 at windows size 6. The results of this study showed that multiple hypothesis testing procedures are related to false positive genomic signals. It is difficult to suggest universally acceptable multiple hypothesis testing and QC measures and their thresholds due to sources of variations between species and within populations. However, additional analytical approaches and studies are needed to evaluate the effects of linkage disequilibrium on the multiple hypothesis testing procedures and QC measures (especially for minor allele frequencies) to GWAS under various scenarios including, but not limited to, level of heritability, linkage disequilibrium, population structure, and population size.

Keywords: *Genome wide association analyses, Multiple hypothesis testing, Quality control procedures*

Sığır Tüberkülozu İçin Çoklu Hipotez Düzeltmesi İle Genom Tabanlı İlişki Analizi

Özet

Genom tabanlı ilişki çalışmaları (GTİÇ) kullanılarak çiftlik hayvanlarının verimleri ile ilişkili tekil nükleotid polymorfizmler (TNP) belirlenebilmektedir. GTİÇ'den elde edilecek sonuçlar hem fenotip hem de genotip veri setlerinin kalitesine bağlı olacaktır. Populasyon tabakası, çoklu hipotez düzeltim yöntemleri ve kalite kontol süreçleri yanlış pozitif (veya negatif) ilişki sonuçlarına yol açabilir. Bu çalışmanın amaçları: bir sığır tüberküloz GTİÇ veri setine 1) değişik kalite ölçütleri ve çoklu hipotez düzeltme yöntemlerinin 2) bazı TNP regresyon yöntemleri ile atasal tabakaların belirlenmesi ve düzeltilmesinin etkilerinin incelenmesidir. Çoklu hipotez düzeltmesi olmadan TNP regresyon modeli ile 2, 7, 8 ve 13. kromozomdan önemli TNP'ler (P<0.001) için belirlendi. Ama çoklu hipotez düzeltmesi Bonferroni düzeltmesi, Hochberg yöntemi ve permutasyon ile gerçekleştirildiğinde genomik sinyallerin önemsiz çıktığı gözlemlendi. Sadece yanlış keşif oranı yöntemi 0.54 seviyesinde zayıf genomik sinyalleri 2, 8 ve 13. kromozomdan belirledi. Çoklu hipotez testlerinde dengesiz bağıntıyı, 2, 4 ve 6 TNP pencere büyüklüğü için modele tanıttık. Pencere büyüklüğü 6 olunca 2, 6, 8 ve 13. kromozomlardan güçlü genomik sinyaller tespit ettik. Bu çalışmanın sonuçları çoklu hipotez test yöntemlerinin yanlış genomik sinyallerin keşfedilmesinde önemli olduğunu ortaya koydu ve doğruladı. Hem türler arası hem de populasyonlar içi varyasyon kaynakları nedeniyle evrensel kalite kontrol ölçütleri önermek oldukça zordur. Bununla birlikte kalıtım derecesi seviyeleri, dengesiz bağıntı, populasyon yapısı ve populasyon büyüklüğü dahil farklı senaryoların varlığında dengesiz bağıntının çoklu hipotez test yöntemlerine etkileri farklı kalite kontrol ölçütleri kullanılarak (özellikle farklı minör alel sıklığı seviyelerinde) GTİÇ için analitik olarak incelenmelidir.

Anahtar sözcükler: *Genom tabanlı ilişki analizi, Çoklu hipotez test yöntemleri, Kalite kontrol yöntemleri*

İletişim (Correspondence)
+90 242 2274400/2507
burakkaracaoren@akdeniz.edu.tr

INTRODUCTION

P values have been heavily used in frequentist statistics (and all other branch of sciences) to evaluate whether null hypothesis (stating there is no treatment effects) true or not. Optimal use of hypothesis testing is an active research area: recently the American Statistical Association published how to correctly use and interpret the p values [1]. P values could be used over time or space. For example Karacaören [2] employed longitudinal p values for modeling time effect in genomic studies. Zaykın et al.[3] used the combined p values over neighboring chromosomal locations (or space) in genome wide association studies (GWAS).

GWAS have been used to detect single nucleotide polymorphisms (SNPs) related to various animal traits. GWAS compare the allele frequencies of cases and controls to determine significant SNPs. Assumptions regarding the genetic architecture of the trait facilitate different statistical models in GWAS. If a trait is assumed to be controlled by many rare variants, a large amount of hypothesis testing must be conducted in piecemeal manner to detect the association [4]. Due to the SNPs depending on chromosomal locations, the naive use of multiple hypothesis testing procedures might lead to a loss of power [5,6]. Therefore, the outcome of GWAS is dependent on the model of multiple hypothesis testing and the quality of both the phenotypic and genotypic datasets [7,8]. False positive (or negative) associations may be obtained due to multiple hypothesis testing process, quality control measures and undetected population structure.

For example, current practices of animal breeding employ assortative mating to obtain higher selection responses in animal production. A GWAS model should take this relatedness structure into account using pedigree and/or genomic information. Several approaches have been proposed for detecting and correcting the effects due to common ancestral clusters using single SNP approaches. Although principal component-based approaches are commonly used in the literature, mixed model-based approaches have also gained popularity recently [9]. Price et al.[10] used principal component analysis to take into account of ancestral population stratifications. Aulchenko et al.[11] suggested conducting genome-wide rapid association using mixed model and regression (GRAMMAR) for phenotypes based on polygenic effects of pedigree structure. Amin et al.[12] extended the GRAMMAR approach by using genomic information instead of pedigree information. Svishcheva et al.[13] extended the original GRAMMAR approach by introducing a gamma factor to adjust the inflation factor. Karacaören [14] defined the original GRAMMAR approach in a Bayesian framework.

Bermingham et al.[15] used GRAMMAR and regional heritability mapping approaches to detect variants using a bovine tuberculosis (BT) dataset. BT is an infectious disease with annual economic costs estimated as €2 billion [16]. However availability (and therefore investigation) of public genomic livestock datasets especially for BT is uncommon. Main reasons are associated with economical and strategical values of the datasets [17]. Since BT dataset included high (617885 SNPs) number of explanatory variables: usage of different multiple hypothesis testing procedures may lead to interesting results. The objectives of this study were to 1) investigate different multiple hypothesis testing procedures with different quality measures and 2) to detect and correct ancestral stratification using different single SNP models for simulated [18] and the BT GWA dataset.

MATERIAL and METHODS

QTL-MAS Simulated DataSet

The quantitative trait locus marker assisted selection (QTL-MAS) simulated data set [18] included 2326 individuals from 10031 biallelic SNPs over 5 chromosomes. The pedigree was simulated according to the half sib family structure. A quantitative trait simulated in association with 37 QTLs. Major QTLs were located on chromomes 1 and 3. We considered mapped QTLs as if the predicted SNPs were located within 5Mb distance from true QTL position. We also investigated number of single mapped QTLs using the same criteria. More details about the dataset could be found at [18].

Phenotypes and Genotypes of the BT Dataset

The BT dataset was obtained from 1151 Holstein -Friesian cows in Northern Ireland [15]. The cases (n=592) and controls (n=559) were defined by single intradermal comparative tuberculin test and abattoir inspection. Individuals were genotyped using BovineHD Illumina Bead 617885 SNPChip. More details about the dataset could be found at [15].

Methods

We applied various quality control measures to the real BT genotypic data set. Due to selection over generations we are not expecting Hardy Weinberg equilibrium in the population. However minor allele frequencies (MAF), individual and genotypic call rates might be important for the outcome of GWAS [19]. Genomic inflation factors could be used to asses if the results of the GWA results are biased using distribution of the test statistics. Multidimensional scaling plots using genomic kinship matrix could be used to investigate genetic outliers in the populations.

Association mapping to detect quantitative trait locus controlling tuberclosis was implemented in R using the various GRAMMAR functions in the GenABEL package [20]. GRAMMAR (raw, genomic control or gamma versions) function uses a two step linear model. In the first step of the GRAMMAR analysis, we estimated the errors for the

phenotype using an animal model as was implemented in GenABEL;

$$y = \mathbf{X}b + \mathbf{Z}a + e \quad \text{(i)}$$

where y contains the observations, b is the fixed effects of age, breed, season of year of and reason of tuberculosis, a is the additive genetic effect, matrices **X** and **Z** are incidence matrices, and **e** is a vector containing residuals.

$$Var\begin{pmatrix} a \\ e \end{pmatrix} \sim N\left[\mathbf{0}; \begin{pmatrix} \mathbf{A}\sigma_a^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{I}\sigma_e^2 \end{pmatrix}\right]$$

For the randomeffects, it is assumed that A is the additive genomic relationship matrfor the animals; I is an identity matrix, σ_a^2 is the additive genetic variance and σ_e^2 is the residual variance. In the second step, assuming a single SNP model for the quantitative trait, we could detect the most significant SNPs using the following model:

$$y = Xf + \eta + e \quad \text{(ii)}$$

where y represents vector of n observations (residuals from (i)), η is intercept, X is a design matrix relating observations with f regression coefficients vector to be estimated, e is a vector of residuals assumed to be normally distributed. Svishcheva et al.[13] extended GRAMMAR approach by introducing gamma factor for adjusting inflation factor. Genetic stratification could also be detected by principal components [10]. Phenotypes of (i) were corrected by principal components for ancestral stratification as was implemented in GenABEL.

We used genomic relationship matrix [21] to take into account of pedigree structure: $\mathbf{A} = WW'/c$ where c is a normalizing constant and $W_{ik} = X_{ik} + 1 - 2p_k$ p_k is the frequency of the 1 allele at marker k. We used the GWA function for association analysis was implemented in R by rrBLUP package [21]. Similar to the GRAMMAR; the GWA function use a mixed linear model to take into account of genetic stratification by genomic relationship matrix. Here the variance of random effect is assumed to be $2\mathbf{A}\boldsymbol{V}_g$ where A is the genomic kinship matrix and $\boldsymbol{V}_g$ is the maximum likelihood estimate of polygenic variance.

Assumptions regarding underling genetic architecture in GWAS is crucial for choosing optimal statistical models for detection of causal variants. Since we assumed that many rare variants are in effect with bovine tuberculosis; we employed a single SNP regression in (ii). However evaluation of null hypothesis for huge number of SNPs may lead to false positive findings. In order to avoid type 1 errors (rejecting of a true null hypothesis); Bonferroni correction, Hochberg's method or false discovery rate approach could be used.

Since huge number of hypothesis testing needs to be done to detect the genomic association; type 1 errors should be controlled as such at α=0.05 level. If we have *m* number of SNPs to test: Bonferroni correction tests each SNPs at the significance level of 0.05/*m*. Different from Bonferroni correction: Hochberg's method compares each *p* value with different significance levels: 0.05/(*m*+1-*k*) using ordering values of *k*. Original false discovery rate approach use critical threshold value of *i**0.05/*m* with ordering values of *i*. Full details of this models could be found in [22,23]. However failing to taken into account of linkage disequilibrium in multiple hypothesis correction by adjacent SNPs may lead to loose of power. Zaykin et al.[3] suggested to use truncated product method for combining p values by chromosomal location of adjacent SNPs in various windows sizes.

RESULTS

QTL-MAS Dataset

QTL-MAS dataset were used for validation of multiple hypothesis testing procedures (Bonferroni, Hochberg and false discovery rate methods) and Zaykin et al.[3] model for QTL mapping. QTLs were mapped by different success rates: both Bonferroni and Hochbergs methods resulted as 0.75 mapping success. However both model did not able to detect 29 (total number of QTLs were 37) single QTLs. Although false discovery rate approach mapping success found to be lower as 0.67: It detected much higher number of single QTLs (20 true QTLs). We combined p values over chromosomal locations using Zaykin et al.[3] approach. Both windows sizes of 4 and 6 gave the same mapping success as 0.74. However Zaykins model predicted highest number of true single QTLs (33 true single QTLs) using both 4 and 6 windows sizes by Bonferroni correction. We noted that Zaykins model detected false positive QTLs from chromosome 5, where there was no QTL on this chromosome, for windows size of 0.

BT Dataset

The Manhattan plot of the transformed (-log) p values using GWA option of rrBLUP [21] revealed strong genomic signals from chromosomes 8 and 13 without any quality control *(Fig. 1)*. We fixed the minor allele frequencies at 0.01 (Quality Control 1, QC1) and 0.05 (Quality Control 2, QC2) and accordingly created two genotypic datasets.

We excluded 10 SNPs with a minor allele frequency <1% and 44,783 SNPs with a minor allele frequency <5%. There was a 0.90 call rate for both animals and SNPs, leaving 617,875 SNPs and 573,102 SNPs in the analyses with 0.01 and 0.05 minor allele frequencies, respectively. We excluded one animal due to a sex chromosome error,

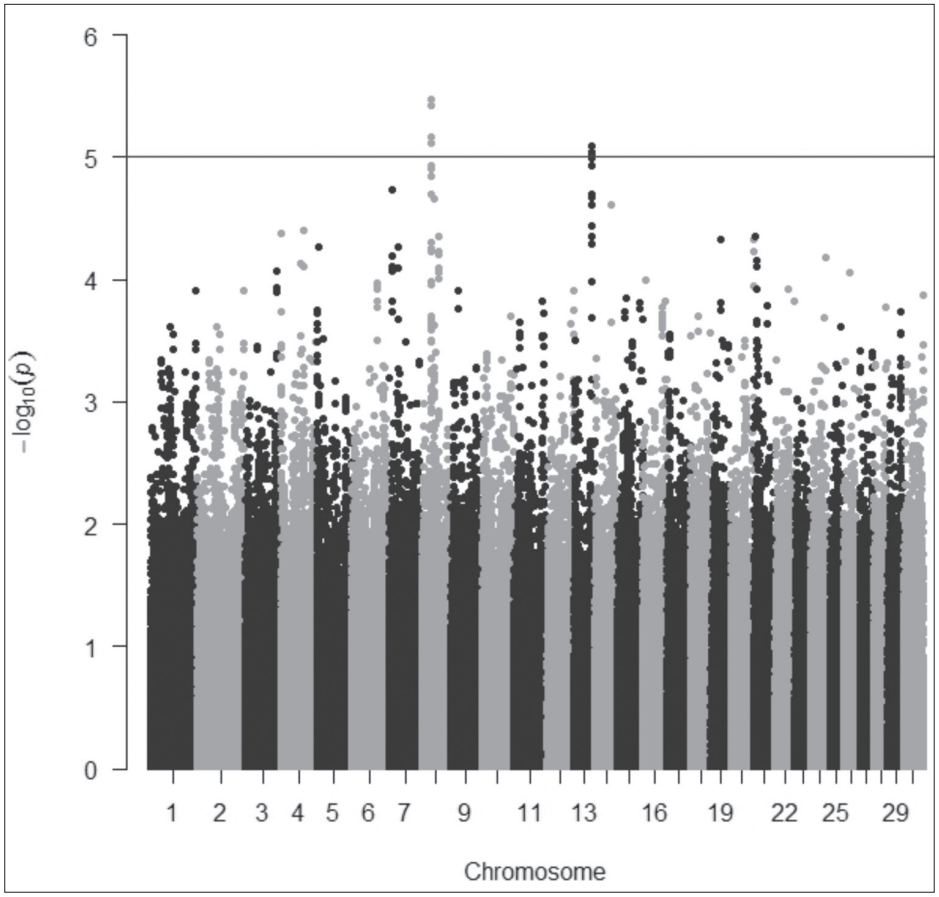

Fig 1. Manhattan plots of -log 10 (P values) using all SNPs (without quality control filters)

Şekil 1. Kalite kontrol süzgeçleri olmadan elde edilen bütün TNP›lere ait -log 10 (p değerleri) için Manhattan şekli

Fig 2. Manhattan plots of -log 10 (P values) using all SNPs with minor allele frequency 0.05, calling rate for animals 0.10, calling rate for genotypes 0.10

Şekil 2. 0.05 minör alel sıklıklı, 0.10 genotip ve hayvan başına çağırım oranlı bütün TNP›lere ait -log 10 (p değerleri) için Manhattan şekli

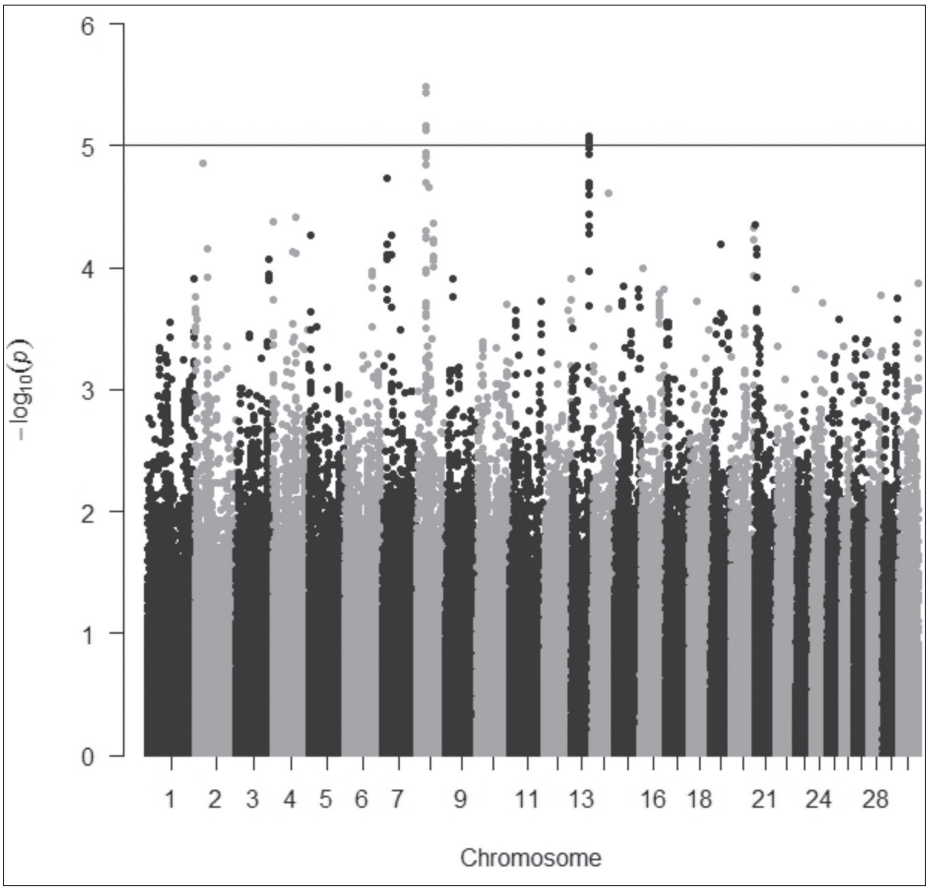

Table 1. *Genome wide association results of QTLMAS dataset using GRAMMAR and different windows sizes (0, 2, 4 and 6)*
Tablo 1. *Farklı pencere büyüklüklü (0, 2, 4 ve 6) GRAMMAR genom tabanlı ilişki sonuçları*

SNP	P-VAL	SIZE_0	SIZE_2	SIZE_4	SIZE_6
5143	1.313E-16	1.23E-12	3.66E-25	6.52E-48	8.97E-71
5144	0.103	1	1.56E-20	9.39E-43	3.04E-70
5145	7.439E-05	0.696142	2.11E-24	5.28E-43	3.32E-70
906	0.3508	1	4.57E-24	8.7E-42	1.5E-68
905	3.501E-12	3.28E-08	1.15E-20	4.98E-41	2.26E-67
904	0.1411	1	6.08E-10	3.02E-44	3.25E-67
5146	0.5324	1	8.34E-10	5.27E-47	4.71E-65
5153	1.196E-06	0.011192	5.99E-15	1.44E-42	1.29E-64
5141	1.172E-05	0.109676	6.31E-36	5.15E-53	2.08E-64
5147	2.487E-14	2.33E-10	4.49E-19	9.51E-47	2.5E-61

Table 2. *Genome wide association results with 0.05 minor allele frequencies of GRAMMAR using genomic control*
Tablo 2. *Genomik kontrol, 0.05 minör alel sıklığı ile GRAMMAR kullanılarak elde edilen genom tabanlı ilişki sonuçları*

Single Nucleotide Polymophism	Chromosome	Location	No of Individuals	Chi Square	P Val	P Val-Permuted
BovineHD0200007460	2	25899036	1057	18.76	1.48E-05	0.93
BovineHD4100005792	7	17622873	1150	18.41	1.78E-05	0.95
BovineHD1300020589	13	71788784	1149	18.30	1.89E-05	0.96
BovineHD1300020586	13	71784332	1146	18.17	2.02E-05	0.96
BovineHD1300020584	13	71782488	1150	18.06	2.14E-05	0.97
BovineHD1300020585	13	71783216	1150	18.06	2.14E-05	0.97
BovineHD1300020590	13	71789620	1150	18.06	2.14E-05	0.97
BovineHD1300020591	13	71791844	1150	17.91	2.32E-05	0.97
BovineHD0800010042	8	33645693	1150	17.76	2.51E-05	0.98
BovineHD4100010384	13	71781867	1139	17.49	2.89E-05	0.99

Table 3. *Principal components corrected genome wide association results with 0.05 minor allele frequencies*
Tablo 3. *0.05 minör alel sıklığı ve temel bileşenler analizi ile düzeltilerek elde edilmiş genom tabanlı ilişki sonuçları*

Single Nucleotide Polymophism	Chromosome	Location	No of Individuals	Chi Square	P Val	P Val-Permuted
BovineHD0800010042	8	33645693	1150	24.62	6.99E-07	0.16
BovineHD1300020589	13	71788784	1149	24.28	8.32E-07	0.18
BovineHD1300020586	13	71784332	1146	24.16	8.86E-07	0.19
BovineHD1300020584	13	71782488	1150	24.04	9.44E-07	0.19
BovineHD1300020585	13	71783216	1150	24.04	9.44E-07	0.19
BovineHD1300020590	13	71789620	1150	24.04	9.44E-07	0.19
BovineHD0800010045	8	33655169	1150	23.70	1.12E-06	0.22
BovineHD1300020591	13	71791844	1150	23.67	1.15E-06	0.23
BovineHD4100010384	13	71781867	1139	23.13	1.51E-06	0.29
BovineHD1300020582	13	71776870	1149	22.63	1.97E-06	0.35

retaining 1150 animals in the analyses. The mean identity by state was found to be 0.72 (0.009) 0.69 (0.008) using QC1 and QC2, respectively. The mean heterozygosis was found to be 0.36 (0.01) and 0.38 (0.01) using QC1 and QC2, respectively. We estimated heritability to be 0.23 and 0.22 using genomic kinship matrix and QC1 and QC2, respectively. Although a multi-dimensional scaling method detected two slightly separated clusters in the population (data not shown), as suggested by Birmingham et al.[15], we retained all of the animals in the dataset for further analyses. Since the results of both the GWA function of rrBLUP *(Fig. 2)* and GenABEL were similar, we opted to discuss only those results obtained from GenABEL.

Table 4. *Genome wide association results with 0.01 minor allele frequencies of GRAMMAR using genomic control*
Tablo 4. *Genomik kontrol, 0.01 minör alel sıklığı ile GRAMMAR kullanılarak elde edilen genom tabanlı ilişki sonuçları*

Single Nucleotide Polymophism	Chromosome	Location	No of Individuals	Chi Square	P Val	P Val-Permuted
BovineHD0200007460	2	25899036	1057	18.71	3.69E-06	0.95
BovineHD1300020589	13	71788784	1149	18.36	4.57E-06	0.97
BovineHD4100005792	7	17622873	1150	18.31	4.69E-06	0.97
BovineHD1300020586	13	71784332	1146	18.22	4.95E-06	0.98
BovineHD1300020584	13	71782488	1150	18.11	5.29E-06	0.98
BovineHD1300020585	13	71783216	1150	18.11	5.29E-06	0.98
BovineHD1300020590	13	71789620	1150	18.11	5.29E-06	0.98
BovineHD1300020591	13	71791844	1150	17.97	5.76E-06	0.99
BovineHD0800010042	8	33645693	1150	17.63	7.06E-06	0.99
BovineHD4100010384	13	71781867	1139	17.56	7.37E-06	0.99

Table 5. *Principal components corrected genome wide association results with 0.01 minor allele frequencies*
Tablo 5. *0.01 minör alel sıklığı ve temel bileşenler analizi ile düzeltilerek elde edilmiş genom tabanlı ilişki sonuçları*

Single Nucleotide Polymophism	Chromosome	Location	No of Individuals	Chi Square	P Val	P Val-Permuted
BovineHD0800010042	8	33645693	1150	24.59	7.10E-07	0.17
BovineHD1300020589	13	71788784	1149	24.26	8.44E-07	0.19
BovineHD1300020586	13	71784332	1146	24.13	9.00E-07	0.20
BovineHD1300020584	13	71782488	1150	24.01	9.58E-07	0.22
BovineHD1300020585	13	71783216	1150	24.01	9.58E-07	0.22
BovineHD1300020590	13	71789620	1150	24.01	9.58E-07	0.22
BovineHD0800010045	8	33655169	1150	23.66	1.15E-06	0.26
BovineHD1300020591	13	71791844	1150	23.63	1.17E-06	0.26
BovineHD4100010384	13	71781867	1139	23.11	1.09E-06	0.32
BovineHD1300020582	13	71776870	1149	22.59	1.01E-06	0.40

The genomic control inflation factors of QC1 for the GRAMMAR genomic control, principal components corrected association and GRAMMAR gamma were 0.87 (7.85×10^{-6}), 1.14 (1.23×10^{-5}) and 0.99 (8.98×10^{-6}), respectively. The genomic control inflation factors of QC2 for GRAMMAR genomic control, the principal components corrected for association and GRAMMAR gamma were 0.87 (9.18×10^{-6}), 1.14 (9.19×10^{-6}) and 0.99 (1.05×10^{-5}), respectively.

Results of the QC1 and QC2 association analyses using, GRAMMAR with genomic control and principal components approaches including permutation tests are reported in *Table 2*, *Table 3*, *Table 4,* and *Table 5*.

DISCUSSION

QTL-MAS Dataset

Investigation of QTL-MAS simulated dataset showed that Bonferroni and Hochberg methods gives conservative results (only 8 QTLS were detected out of 37) as was also concluded by Johnson et al.[24]. Fu et al.[6] showed advantaged of using linkage disequilibrium information in multiple hypothesis testing. As was also showed in *Table 1*: taking into account of linkage disequilibrium structure leads to higher p values proportional to the windows sizes. On the basis of these observations we found that: the Zaykins model built from different windows sizes found the highest number of true QTLs (33 out of 37). Similarly Hu et al.[25] showed by simulation that grouping of p values leads to higher statistical power in the genetic association studies.

BT Dataset

We found that the genomic control inflation factors were similar for each association model using QC1 and QC2. The GRAMMAR gamma inflation factors were approximately 1, which indicates that this method is unbiased compared with other correction methods. Bermingham et al.[15] also noted a minimal increase in the lambda values, similar to the results of GRAMMAR with gamma inflation factors.

Since Bermingham et al.[15] possibly used GRAMMAR with gamma inflation factors, we did not reproduce the same results in our study. The results of the QC1 and QC2 association analyses using GRAMMAR with genomic

control and principal component approaches including permutation tests are reported in *Tables* 2-5. We detected SNPs from chromosomes 2, 7, 8 and 13 at a significance level of P<0.001 without correcting for multiple hypothesis testing. However, after the Bonferroni correction, Hochberg's method and the permutation test for multiple hypothesis correction *(Tables 2-5)*, the genomic signals became non-significant. Only the false discovery rate approach detected weak signals (at a level of 0.54) from chromosomes 2, 8 and 13.

Type 1 errors would increase without correcting for multiple hypothesis testing. However, traditional correction models do not take into account dependency among hypotheses. In GWAS, one of the sources for the correlated hypothesis may be linkage disequilibrium. In addition, failing to take into account linkage disequilibrium among adjacent SNPs may lead to a reduction of power. We used the Zaykin et al.[3] model to take into account the effect of linkage disequilibrium on the multiple hypothesis testing procedures by combining adjacent SNP test statistics with window sizes of 2, 4 and 6. We detected strong genomic signals from chromosome 13 (-log(p)=90.33), chromosome 8 (-log(p)=51.34), chromosome 16 (-log(p)=49.47) and chromosome 2 (-log(p)=44.77) at a window size of 6. However, the test statistics will increase as the window size increases using the approach of Zaykin et al.[3]. Although top SNPs have been found to be the same irrespective of the windows size, larger windows sizes may lead to anti-conservative results [26].

The results of this study have shown that multiple hypothesis testing procedures are related to false positive genomic signals. Although the level of minor allele frequencies did not make a difference in terms of genomic signals *(Tables 2-5)*, these results cannot be generalized [7]. It is difficult to suggest universally acceptable multiple hypothesis testing [24] and quality control measures and their thresholds due to sources of variations between species and within populations. However, additional analytical approaches and studies are necessary to evaluate the effects of linkage disequilibrium on the multiple hypothesis testing procedures and QC measures (especially for minor allele frequencies) to GWAS under various scenarios including, but not limited to, the level of heritability, linkage disequilibrium, population structure and population size.

REFERENCES

1. Wasserstein RL, Lazar NA: The ASA's statement on p-values: Context, process, and purpose. *Am Stat*, 70, 129-133, 2016. DOI: 10.1080/00031305.2016.1154108

2. Karacaören B: A bayesian random walk approach for mapping dynamic quantitative trait. *J App Non Dyn*, 5, 105-115, 2016. DOI: 10.5890/JAND.2016.03.008

3. Zaykin DV, Zhivotovsky LA, Westfall PH, Weir BS: Truncated product method for combining P-values. *Genet epid*, 22, 170-185, 2002. DOI: 10.1002/gepi.0042

4. Sham PC, Purcell SM: Statistical power and significance testing in large-scale genetic studies. *Nat Rev Genet*, 15, 335-346, 2014. DOI: 10.1038/nrg3706

5. Kang G, Ye K, Liu N, Allison DB, Gao G: Weighted multiple hypothesis testing procedures. *Stat Appl Genet Molec Biol*, 8, 1-22, 2009. DOI: 10.2202/1544-6115.1437

6. Fu G, Saunders G, Stevens J: Holm multiple correction for large-scale gene-shape association mapping. *BMC Genet*, 15 (Suppl. 1): S5, 2014. DOI: 10.1186/1471-2156-15-S1-S5

7. Tabangin ME, Woo JG, Martin LJ: The effect of minor allele frequency on likelihood of obtaining false positives. *BMC Proc*, 3 (Suppl. 7): S41, 2009. DOI: 10.1186/1753-6561-3-S7-S41

8. Chen G, Lee SH, Robinson MR, Trzaskowski M, Zhu Z, Winkler TW, Day FR, Croteau-Chonka DC, Wood AR, Locke AE, Kutalik Z, Loos RJF, Frayling TM, Hirschhorn JN, Yang J, Wray NR, The Genetic Investigation of Anthropometric Traits (GIANT) Consortium, Visscher PM: Across-cohort QC analyses of GWAS summary statistics from complex traits. *Eur J Hum Genet*, 2016 (In press). DOI: 10.1038/ejhg.2016.106

9. Eu-ahsunthornwattana J, Howey RA, Cordell HJ: Accounting for relatedness in family-based association studies: Application to genetic analysis workshop 18 data. *BMC Proc*, 8, (Suppl. 1): S79, 2014. DOI: 10.1186/1753-6561-8-S1-S79

10. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D: Principal components analysis corrects for stratification in genome-wide association studies. *Nat genet*, 38, 904-909, 2006. DOI: 10.1038/ng1847

11. Aulchenko YS, De Koning DJ, Haley C: Genomewide rapid association using mixed model and regression: A fast and simple method for genomewide pedigree-based quantitative trait loci association analysis. *Genetics*, 177, 577-585, 2007. DOI: 10.1534/genetics.107.075614

12. Amin N, Van Duijn CM, Aulchenko YS: A genomic background based method for association analysis in related individuals. *PloS one*, 2, e1274, 2007. DOI: 10.1371/journal.pone.0001274

13. Svishcheva GR, Axenovich TI, Belonogova NM, van Duijn CM, Aulchenko YS: Rapid variance components-based method for whole-genome association analysis. *Nat Genet*, 44, 1166-1170, 2012. DOI: 10.1038/ng.2410

14. Karacaören B: Admixture mapping of growth related traits in F 2 mice dataset using ancestry informative markers. *J Bioinform Comput Biol*, 12, 2014. DOI: 10.1142/S0219720014410108

15. Bermingham ML, Bishop SC, Woolliams JA, Pong-Wong R, Allen AR, McBride SH, Ryder JJ, Wright DM, Skuce RA, McDowell SWJ, Glass EJ: Genome-wide association study identifies novel loci associated with resistance to bovine tuberculosis. *Heredity*, 112, 543551, 2014. DOI: 10.1038/hdy.2013.137

16. Garnier T, Eiglmeier K, Camus JC, Medina N, Mansoor H, Pryor M, Duthoy S, Grondin S, Lacroix C, Monsempe C, Simon S, Harris B, Atkin R, Doggett J, Mayes R, Keating L, Wheeler PR, Parkhill J, Barrell BG, Cole ST, Gordon SV, Hewinson RG: The complete genome sequence of *Mycobacterium bovis. Proc Natl Acad Sci USA*. 100, 78-77, 82, 2003. DOI: 10.1073/pnas.1130426100

17. Cleveland MA, Hickey JM, Forni S: A common dataset for genomic analysis of livestock populations. *G3: Genes Genom Genet*, 2, 429-435, 2012. DOI: 10.1534/g3.111.001453

18. Szydlowski M, Paczynska P: QTLMAS 2010: Simulated dataset. BMC Proc, 5 (Suppl 3): S3, 2011. DOI: 10.1186/1753-6561-5-S3-S3

19.Turner S, Loren LA, Yuki B, Christopher SC, Dana CC, Andrew T. C, Mariza de A, Kimberly FD, Jonathan LH, Geoffrey H, Gail J, Lan J, Iftikhar JK, Rongling L, Hua L, Teri AM, Martha M, Catherine AM, Andrew NM, Daniel BM, Justin EP, Elizabeth WP, Luke VR, Russell AW, Rebecca LZ, Marylyn DR : Quality control procedures for genome-wide association studies. *Curr prot hum genet*, 1-19, 2011. DOI: 10.1002/0471142905.hg0119s68

20. Aulchenko YS, Ripke S, Isaacs A, Van Duijn CM: GenABEL: An R library for genome-wide association analysis. *Bioinformatics*, 23, 1294-1296, 2007. DOI: 10.1093/bioinformatics/btm108

21. Endelman JB: Ridge regression and other kernels for genomic

selection with R package rrBLUP. *Plant Genome*, 4, 250-255, 2011. DOI: 10.3835/plantgenome2011.08.0024

22.Balding DJ: A tutorial on statistical methods for population association studies. *Nat Rev Genet*, 7, 781-791,2006. DOI: 10.1038/nrg1916

23. Goeman JJ, Solari A: Multiple hypothesis testing in genomics. *Stat Med,* 33, 1946-1978, 2014. DOI: 10.1002/sim.6082

24. Johnson RC, Nelson GW, Troyer JL, Lautenberger JA, Kessing BD, Winkler CA, O'Brien SJ: Accounting for multiple comparisons in a genome-wide association study (GWAS). *BMC genomics*, 11, 1, 2010. DOI: 10.1186/1471-2164-11-724

25. Hu X, Zhang W, Zhang S, Ma S, Li Q: Group-combined P-values with applications to genetic association studies. *Bioinformatics*, 2016 (In press). DOI: 10.1093/bioinformatics/btw314

26. Lin WY, Lou XY, Gao G, Liu N: Rare variant association testing by adaptive combination of P-values. *PloS one*, 9 (1): e85728, 2014. DOI: 10.1371/journal.pone.0085728