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Power of Variance Component Linkage Analysis to Identify Quantitative Trait Locus in Chickens

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ABSTRACT

A crucial first step in the planning of any scientific experiment is to evaluate an appropriate sample size to permit sufficient statistical power to detect the desired effect. In this study, we investigated the optimal sample size of quantitative trait locus (QTL) linkage analysis for simple random sibship samples in pedigreed chicken populations, under the variance component framework implemented in the genetic power calculator program. Using the program, we could compute the statistical power required to achieve given sample sizes in variance component linkage analysis in random sibship data. For simplicity, an additive model was taken into account. Power calculations were performed to relate sample size to heritability attributable to a QTL. Under the various assumptions, comparative power curves indicated that the power to detect QTL with the variance component method is highly affected by a function of the effect size of QTL. Hence, more power can be achievable for QTL with a larger effect. In addition, a marked improvement in power could be obtained by increasing the sibship size. Thus, the use of chickens is advantageous regarding the sampling unit issue, since desirable sibship size can be easily obtained compared with other domestic species.

(**Key words :** Chicken, Linkage, Power, Quantitative trait locus, Sample size, Sibship, Variance component)

INTRODUCTION

Regarding economically important traits, which are inexpensive to measure in poultry industry, selective breeding has been tremendously successful in improving genetic potentials with the aid of the relatively short generation times, low environmental variations, and capability to generate large pedigrees (Rowe, 2008). The current advent of DNA technologies and statistical genetic methodologies has made it possible to detect quantitative trait loci (QTL) or quantitative trait nucleotides (QTN) providing genomic information for marker-assisted selection or gene-based selection to achieve the genetic improvement of economic traits, which are expensive to record (Goddard and Hayes, 2009). Among them, the variance component (VC) approach for linkage analysis is a robust method to localize QTL and estimate their effects in human and commercial domestic animal populations. This robustness is due to the ease of the method to cope with complex pedigree and arbitrary size (Almasy and Blangero, 2010; Pérez-Enciso and Misztal, 2011).

Although high density single nucleotide polymorphism (SNP) chip panels provide genome-wide coverage for association studies and genome-enabled selection experiments in chickens (Gu et al., 2011; Wolc et al., 2011), VC QTL linkage analysis is still thought to be a productive method of choice in combination with currently available customized SNP chips, which we can select SNP markers in the regions or genes of interests for the chip, by employing a two-step approach. In the 1st step, whole genome regions are scanned by microsatellite marker based linkage analysis using reference family. Positional candidate gene association analysis in the most promising linkage regions are then conducted using the customized SNP chip in the same family. This will reduce the number of SNP markers for testing association and thereby remove wastage of resources on markers unlikely to be associated with phenotype of interests. Therefore, results of linkage can be used as

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informative priors on the identification of genes responsible for the economically important phenotypic variations. In addition, this two step approach can reduce the multiple testing problems in the genome-wide association studies by the focused positional candidate gene analysis in the promising genome regions identified by linkage analysis (Almasy and Blangero, 2009).

The appropriate use of VC linkage analysis can be facilitatedby projecting realistic scenarios of the performance of the method with certain data structure and size (Williams and Blangero, 1999). Hence, statistical power analysis has been invaluable for investigating operating parameters of linkage analysis. Here, we present results of power analysis in the frame of VC linkage analysis using the genetic power calculator program (Purcell et al., 2003).

MATERIALS AND METHODS

The following factors were taken into account in this power study of VC linkage analysis:

1. α

To declare the identification of QTL, it is necessary to set up an appropriate level of α, the probability of false-positive error (Type I error, *P*-value). We set up the α level as 0.0001 for this power analysis of VC linkage analysis (Williams and Blangero, 1999). This level of α corresponds to a logarithm of odds (LOD) score of 3 indicating the odds of 1000: 1 that the linkage being identified did not occur by chance.

2. 1-β

β, the probability of false-negativeerror (Type II error), can be used to obtain statistical power (i.e., 1-β). The statistical power of a study is the probability of rejecting the null when it is, in fact, incorrect. For linkage studies, the power can be considered as the probability of correctly identifying a genuine linkage. A statistical power of 80% was considered as a minimally appropriate level of the power.

3. Sample size

The sample size is the number of subjects required to perform the VC linkage analysis. Sample sizes of 200, 600, and 1000 were considered as sample sizes for calculating the power.

4. Sibship size

The sibship size is the number of offspring within a family. Sibsize of 2, 4, 6, and 8 were taken into account for calculating the power.

5. QTL effect size

The effect size was considered to be the proportion of phenotypic variance explained by the QTL (QTL_{h^2}).

Under the various assumptions, the genetic power calculator (GPC) program at http://pngu.mgh.harvard.edu/~purcell/gpc/ was used to investigate the optimal sample size of QTL linkage analysis for simple random sibship samples, under the VC framework (Purcell et al., 2003).

For simplicity, an additive model was taken into account. The average marker distance, expressed in terms of Morgan between markers, was 0.20 Morgan. After the transformation to recombination fraction using the Kosambi mapping function, the recombination fraction was 0.19.

RESULTS AND DISCUSSION

1. Power

Fig. 1 shows the comparative curves of power vs. QTL heritabilities (i.e., fractions of phenotypic variance explained by QTL, QTL_{h^2}) under various assumptions. The power curves corresponded to various sample sizes (n=200, 600, and 1000) and sibsizes (i.e., sampling unit; n=2, 4, 6, and 8). If a significant QTL explaining 26.3% of phenotypic variance (QTL_{h^2}), given its sample size with n = 600 and sibsize with n = 8 is detected, Fig. 1 shows that we can achieve 80% power. However, given n = 1000 and sibsize = 6 with a 26.3% of QTL_{h^2}, power was lower even for a sample size of n = 600 with sibsize = 8. Thus, improvements in power can be achieved by increasing the size of sampling unit. Fig. 1 also demonstrated that the power to identify QTL with the VC approach is a function of the QTL_{h^2}. In terms of effect size of QTL, a much larger sample size is necessary to detect QTL explaining a minor effect (e.g., $QTL_{h^2} < 10\%$) size.

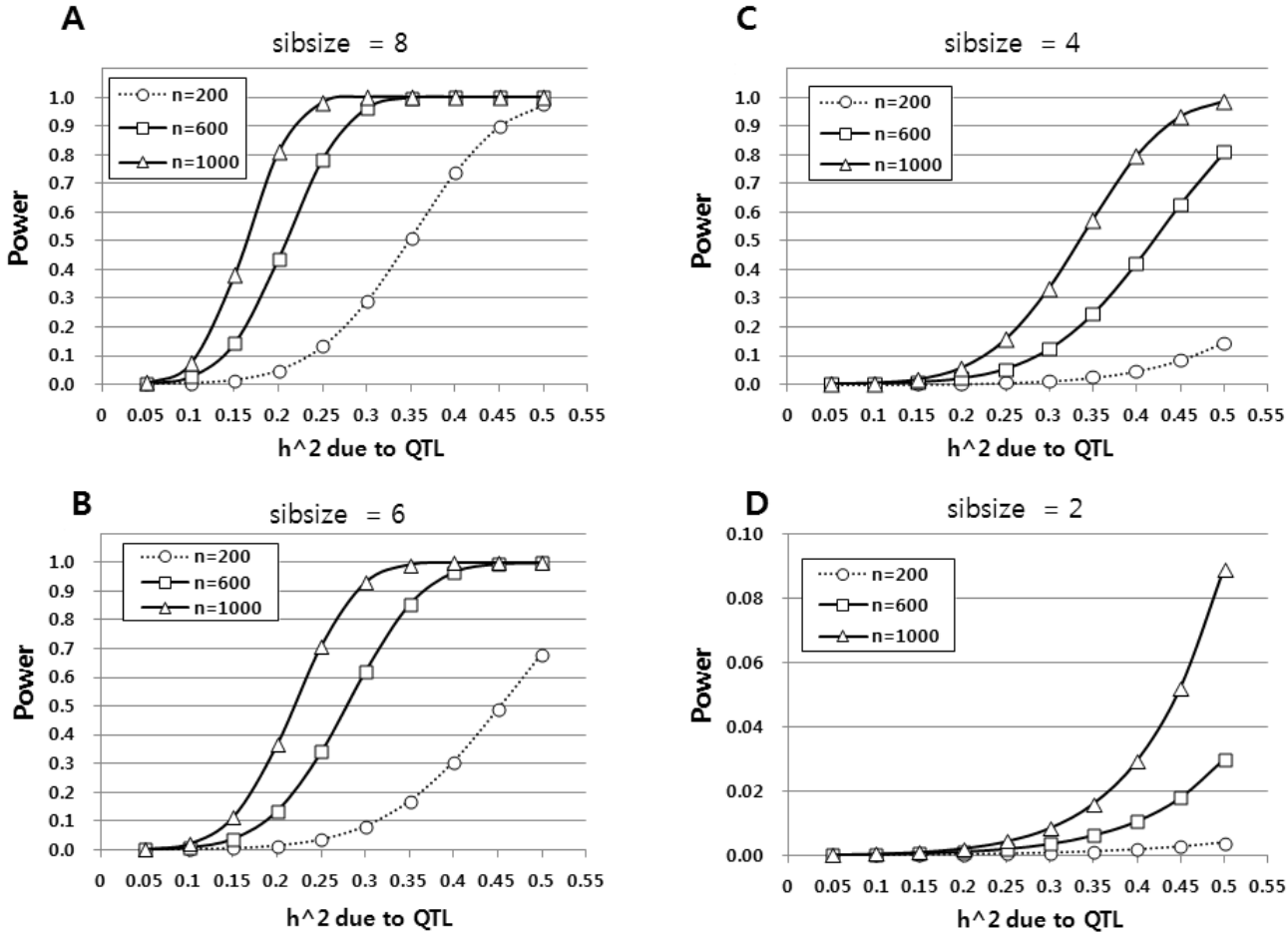

Fig. 1. Comparative power curves for various sizes of sample and sibships in linkage analysis to identify QTL. Panels A, B, C, and D are with different sibsize.

2. Sample sizes

To address the issue of sample size, we performed the sample size estimation to detect a QTL under different sibsizes (Table 1). The effect size of a QTL (i.e., QTL_{h^2}) was also considered for the sample size calculation. In order to achieve 80% statistical power for detecting a significant QTL (i.e., LOD = 3.0, nominal *P*-value = 0.0001) with QTL_{h^2} = 0.25, our power calculation indicated that a sample size of 616 would be necessary given a sibsize n = 8. Hence, a sample size of approximately 620 would not be too small for a study aimed at detecting a linkage with the condition described above.

In QTL analysis, one of the most common experimental designs is an F_2 intercross between divergently selected breeds utilizing between breeds genetic difference. Compared to other experimental designs (e.g., extended family design, F_1 nuclear family design) for QTL linkage analysis, the F_2 design requires apparently fewer numbers of animals because we can assume that all F_1 animals are heterozygous at major QTL; thereby, the power to detect QTL can be maximized (Haley and Andersson, 1997). However, although previous studies have shown the efficiency in detecting QTL that accounts for genetic differences between divergent lines, studies using the F_2 intercross design have provided no practical insight into whether these QTL segregate within current commercial populations that have been under intensive selection. For the successful implementation of QTL information into selective breeding programs, segregation of QTL needs to be confirmed within the selection populations (De Koning et al., 2003). To verify the presence of QTL within a commercial population, VC linkage analysis based on within breed genetic variability can be a method of choice because it can utilize the existing pedigree structure

Table 1. Estimated sample size required for 80% statistical power to detect QTL in VC linkage analysis

h^2 of QTL	Sample size			
	sibsize = 8	sibsize = 6	sibsize = 4	sibsize = 2
0.05	16260	30220	75230	449600
0.10	4056	7524	18710	111700
0.15	1783	3303	8213	49190
0.20	985	1823	4538	27300
0.25	616	1139	2838	17170
0.30	416	769	1917	11670
0.35	297	546	1364	8358
0.40	219	403	1007	6213
0.45	167	306	764	4746
0.50	130	237	592	3701

and data recording of breeding populations.

The statistical power to identify QTL with the VC method is mainly a function of QTL_{h^2}, and more power can be achievable for QTL with a larger effect. Moreover, we may not exclude the possibility of over simplification of the genetic architecture of quantitative traits, if we generalize that QTL explains only a minor proportion of quantitative phenotypic variances. A marked improvement in power can be obtained by increasing the size of the sibship. As the size of the sibship increases, the power approaches the levels that are achievable. Interestingly, the increased sibship size provides more statistical power, regardless of the size of QTL_{h^2}. Hence, the use of chicken population is an advantage regarding the sampling unit issue, since we can obtain a desirable sibship size with ease compared with other domestic species. Recently, the QTL project has been launched using 88 F_0 and 595 F_1 birds from five lines of Korean native chicken (Black, Grey-Brown, Red-Brown, White, and Yellow-Brown) (Hoque et al., 2010; Park et al., 2012; Choi et al., 2013). The main aim of this project is to identify QTL and their associated positional candidate genes for economically important traits based on variance component linkage analysis. The result of power analysis was used to guide the determination of the sample size of the project.

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