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Method

A random model for mapping imprinted quantitative trait loci in a structured pedigree: An implication for mapping canine hip dysplasia

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Abstract

Genetic imprinting may have played a more notable role in shaping embryonic development of plants, animals, and humans than previously appreciated. Quantitative trait loci that are imprinted (*i*QTL) exert monoallelic effects, depending on the parent of origin, which is an exception to the laws of Mendelian genetics. In this article, we present a modified random effect-based mapping model to use in a genome-wide scan for the distribution of *i*QTL that contribute to genetic variance for a complex trait in a structured pedigree. This model, implemented with the maximum likelihood method, capitalizes on a network of relatedness for maternally and paternally derived alleles through identical-by-descent sharing, thus allowing for the discrimination of the genetic variances due to alleles derived from maternal and paternal parents. The model was employed to map *i*QTL responsible for canine hip dysplasia in a multihierarchical canine pedigree, founded with seven greyhounds and six Labrador retrievers. Of eight significant QTL detected, three, located on CFA1, CFA8, and CF28, were found to trigger significant parent-of-origin effects on the age of femoral capital ossification measured at the left and right hips of a canine. The detected *i*QTL provide important candidate regions for fine-mapping of imprinted genes and for studying their structure and function in the control of complex traits.

Keywords: Random-effect model; Imprinting quantitative trait loci; Canine hip dysplasia; Parent-of-origin effect

As a consequence of epigenetic modification, imprinted genes display differential expression between maternal and paternal alleles, causing "parent-of-origin" effects on the expression of a phenotypic trait [1]. Increasing evidence has been observed from animal and human studies that imprinted genes may influence cancer, obesity, diabetes, behavior, and cognitive functioning [2–4]. Thus far, more than 70 imprinted genes have been identified to play a pivotal role in shaping embryonic development in mammals, including three well-documented examples, (1) the paternally expressed insulin-like growth factor-2 (*IGF2*) [5,6], (2) the maternally expressed cell receptor for *IGF2* (*Igf2r*) [7], and (3) the *Xist* gene, which inactivates the expression of the paternally derived X chromosome in a female cell [8]. With the advent of new experimental studies and analytical tools, it is possible that new imprinted genes can be characterized and their role in disease susceptibility can be better understood.

The effects of imprinted quantitative trait loci, or *i*QTL, can be estimated in controlled crosses of outbred parents [9–12]. However, genetic differences detected by such a fixed-effect model may result from the allelic heterozygosity of the parents rather than the imprinted effect of *i*QTL [13]. Also, it is difficult for the fixed-effect model to specify and estimate the effects of QTL for heterozygous species because the number of the QTL alleles is unknown. Multiallelic markers, such as microsatellites, have proven to be powerful for studying the genetic architecture of heterozygous populations. Multiple alleles of

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these markers, while used in QTL mapping by a fixed-effect model, are collapsed into two categories, one being the commonest allele and the second being the collection of all the other alleles. Such a reduction from multiallelic to "biallelic" markers may affect the power for QTL detection because the segregating information of multiallelic markers is not fully used. For a heterozygous population genotyped by multiallelic markers, a random-effect model has been considered for QTL mapping by estimating the genetic variance contributed by QTL alleles [14].

The motivation of this study was to modify the random-effect model to map *i*QTL that segregate in a complex structured pedigree. The model implements parent-specific identical-by-descent (IBD) sharing into the likelihood constructed for QTL mapping, allowing for the estimation and testing of the additive genetic variance due to maternally or paternally derived QTL alleles. In a few previous publications, the idea of IBD sharing has been used to identify imprinted genes for sibship data [15,16] or structured pedigrees [17,18]. However, none of them have integrated this idea into linkage mapping to provide a genome-wide scan for the existence and distribution of *i*QTL. In this article, we will employ the random-effect model to map *i*QTL for canine hip dysplasia (CHD) in a multigenerational outbred canine pedigree, as used by Todhunter et al. [19,20].

CHD is a developmental orthopedic disease in which abnormal formation of the hip leads to looseness of the hip joints, causing cartilage damage [19]. CHD is a multifactorial trait that is controlled by an array of interacting genes as well as by environmental factors. CHD can be described by different morphological and anatomical characteristics. As an example, we will demonstrate the usefulness of the random *i*QTL mapping model by mapping the emergence ages of hip dysplasia measured as femoral capital ossification. The statistical properties of the model are investigated by simulation studies.

Results

A complex multihierarchical outbred canine pedigree has been used for the genetic mapping of CHD. This pedigree was initiated with seven greyhound and six Labrador retriever founders in an attempt to maximize phenotypic ranges in CHD-related traits [20]. The pedigree is composed of 148 dogs allocated into 16 different families of various sizes (Fig. 1). Based on the most recent version of the integrated canine genome map [21], a set of 240 microsatellite markers that cover about 2000 cM or 80% of the canine genome was genotyped. Of these markers, 166 were highly informative (heterozygosity >0.59), 58 were moderately informative ($0.3<$heterozygosity<0.59) and 16 were uninformative (heterozygosity <0.3) [22].

For dysplastic dogs, the metrics of left and right hips may be controlled by different genetic factors [23]. In this study, our analysis focused on one CHD trait, i.e., the emergence age of hip dysplasia measured as femoral capital ossification (OSS), which was compared for genetic control between the left and the right side. In this pedigree, OSS at the left and right, both following an approximately normal distribution, were averaged as 10.82 ± 3.14 and 10.84 ± 3.21, respectively. Whether different QTL are involved in the control of dysplasia at the right compared to the left canine hip was tested, although the overall means were similar at the two sides.

The OSS phenotypes were associated with the marker genotypes by using two different models, the traditional Mendelian model, in which the genetic variances due to maternal (σ^2_{aM}) and paternal alleles (σ^2_{aP}) are constrained to be identical, and the imprinting model, in which these two genetic variances are assumed to be different. A grid approach assuming the underlying QTL at every 2 cM within a tested marker interval was used to scan for the existence of QTL throughout the canine genome. For each marker interval being scanned, those individuals that miss either marker or phenotypic data were excluded from analyses. The excluded individuals accounted for less than 10% of the full sample size for most marker intervals. The comparison between the imprinting and the Mendelian models can be used to test the significance of the imprinting effect of an *i*QTL. Multiple permutation tests were performed to determine the genome-wide critical threshold for the detection of a significant QTL by shuffling the OSS phenotypes among dogs. The maximum values of the log-likelihood ratios (LR) throughout the genome were estimated for the shuffled data. The 99th percentiles of the empirical distribution of the LR values under the null hypothesis of no QTL in terms of the imprinting model were 6.10 and 6.43 for the left and right OSS, respectively.

Eight QTL for OSS were detected on different canine chromosomes (CFA) at the 1% significance level (Table 1). Fig. 2 illustrates the peaks of the genome-wide log-likelihood ratio profile indicating the maximum likelihood estimates (MLEs) of the QTL positions from the imprinting model under Hypothesis (7). Of the QTL detected, four, detected on CFA1, CFA5, CFA8, and CFA28, are the "generalist" QTL that affect OSS for both the left and the right sides of a hip (Table 1). The other QTL are the "specialist" QTL that affect OSS at only one side, with two, on CFA9 and CFA17, being responsible for the left side and two, on CFA3 and CFA22, being responsible for the right side. Theoretically, the sum of the genetic variance contributed by a QTL (σ^2_a) and the polygenic variance (σ^2_g) should be consistent among the QTL detected. This does not exactly hold because different missing patterns of data occur for the markers that are associated with the detected QTL.

All the detected QTL were further tested for their imprinted effects by comparing the imprinting against the Mendelian model (Hypothesis (9), as shown under Materials and methods). A QTL is regarded as imprinted if there is a significant difference between the genetic variances for completely maternally and paternally derived alleles. Further tests were performed to judge whether the detected *i*QTL is imprinted maternally (Hypothesis (10)) or paternally (Hypothesis (11)). It was found that three pleiotropic QTL, on CFA1, CFA8, and CFA28, for both hip sides display an imprinting effect, whereas the other QTL do not (Table 1). The CFA8 QTL is paternally imprinted for both hip sides of OSS, whereas the CFA28 QTL is

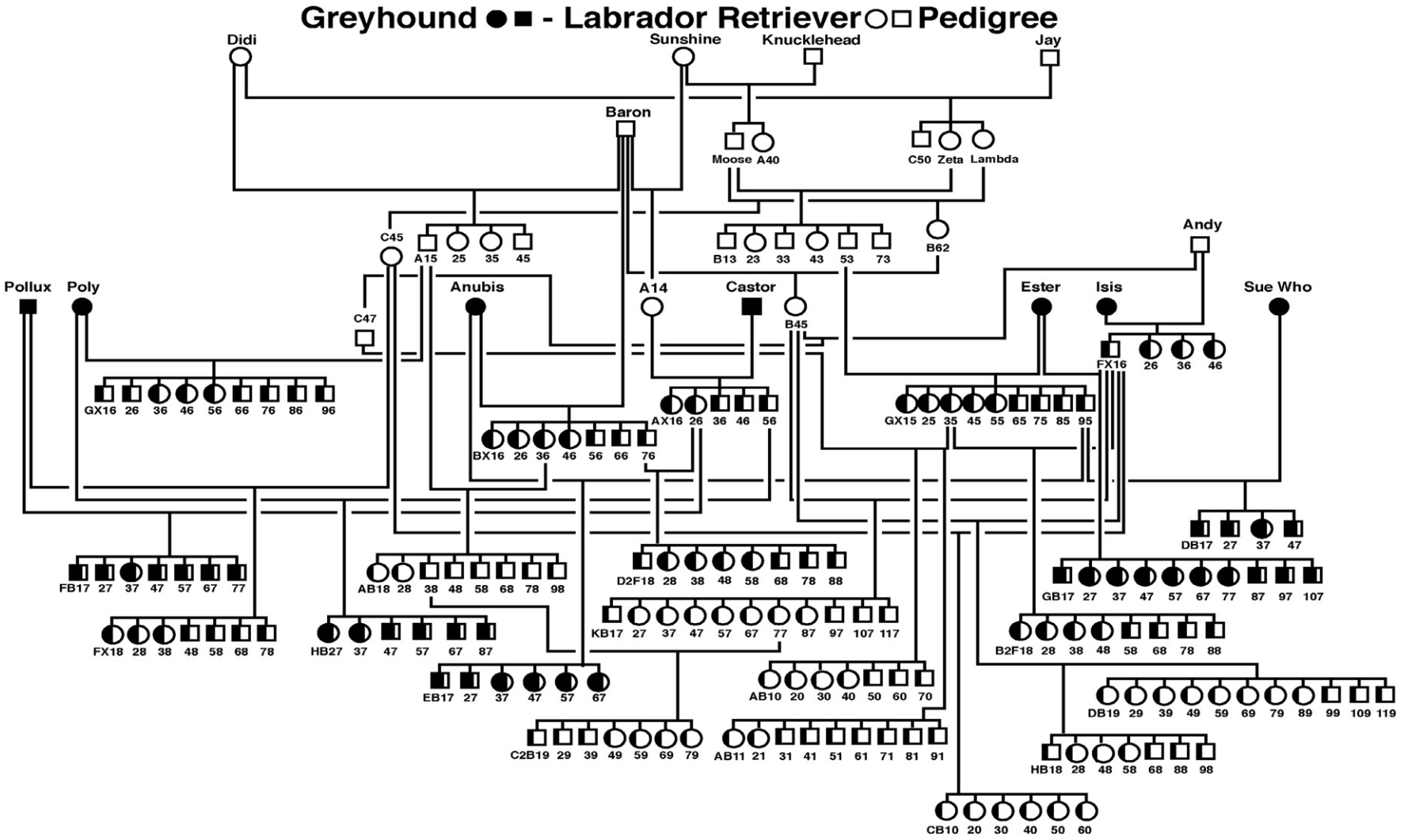

Fig. 1. Diagram of an outbred dog pedigree. Squares and circles represent males and females, respectively. Filled and open portions of each symbol represent the proportion of greyhound and labrador retriever alleles, respectively, possessed by that dog.

Table 1
MLEs of QTL position ang genetic variance for OSS measured at the left (L) and the right (R) side of a canine hip and QT significance tests under the Mendelian and imprinting models

CFA	Marker interval	Trait	Mendelian			Imprinting				Direction
			σ_a^2	σ_g^2	LR	σ_{aM}^2	σ_{aP}^2	σ_g^2	LR_I	
General QTL										
1	C01.673–FH2313	L	7.61	0.00	6.1	0.01	6.25	0.01	4.6	Maternal
		R	11.05	0.00	20.5	0.01	5.11	0.78	2.8	—
5	CPH14–C05.377	L	1.59	5.75	7.1	0.32	1.52	4.74	0.2	—
		R	2.07	4.35	13.1	0.56	2.27	3.27	0.4	—
8	FH2138–REN288F11	L	6.72	0.00	14.3	5.29	0.01	0.02	5.7	Paternal
		R	6.68	0.00	20.3	5.10	0.01	0.01	7.0	Paternal
28	REN309N19–REN146G17	L	6.32	0.00	11.2	0.01	5.89	0.01	4.0	Maternal
		R	4.36	0.00	18.9	0.03	4.85	0.15	4.4	Maternal
Special right QTL										
9	REN75M10–FH2263	L	4.21	1.74	9.9	3.45	0.01	2.66	2.6	—
17	REN50B03–FH2321	L	5.00	1.77	6.7	3.45	1.90	0.76	0.1	—
Special right QTL										
3	FH2107–PES12	R	3.06	2.50	6.5	2.44	2.95	1.14	0.0	—
22	FH2109–REN107H05	R	3.62	2.12	7.9	3.12	0.01	2.78	1.8	—

The imprinting model assumes that maternally and paternally derived alleles contribute differently to the genetic variance. LR and LR_I are the log-likelihood ratios calculated under Hyphotheses (7) and (11) respectively.

maternally imprinted. The CFA1 QTL is maternally imprinted only for the left hip. No evidence for imprinting was found for any of the QTL that affect only one side.

To investigate the statistical behavior of the imprinting model, we performed three different scenarios of simulation as follows:

Scenario 1

In this scenario, we mimic the structure of Fig. 1's canine pedigree by simulating the same sample size (148) allocated between 16 families with various sizes according to the actual pattern of the pedigree. The parameters, overall mean, maternally and paternally derived additive genetic variances, polygenic variance, and residual variance, used to simulate the normally distributed phenotypic data of a quantitative trait were set in a range of each of their estimates obtained from the canine pedigree.

To reduce computational burden, only three ordered multi-allelic markers with two, three, and five allelic states, respectively, spaced by 20 cM, on a linkage group were simulated. A QTL with two alleles was assumed at 4 cM from the second marker in the second marker interval. Assuming equal allele frequency at each marker and QTL, 100 founders were simulated, from which 7 founders were randomly sampled as maternal parents and 6 founders as paternal parents to produce the structured families as shown in Fig. 1. The QTL genotypes segregating in each family were simulated from marker genotypes based on the marker–QTL relationship in terms of IBD sharing.

Table 2 lists the results for the MLEs of QTL parameters averaged over 100 simulation replicates. The imprinting model can detect the existence of the QTL. The QTL position and genetic variances due to the QTL and polygenes can be reasonably estimated. The data were further subjected to analyses and tests for the imprinting effects of the QTL by comparing the imprinting against the Mendelian model. The estimates of genetic parameters including the genetic variances due to maternally and paternally derived alleles of the QTL and polygenes can be well estimated for both simulation strategies.

To show whether the structured pedigree like Fig. 1 is more advantageous for the detection of *i*QTL, we simulated the same number of independent families with results tabulated in Table 2. Relative to structured families, independent families increase, by one-half, the sampling errors of the MLEs of the imprinting genetic variance and residual variance. Also, the structured pedigree as an actual case for the canine pedigree used (Fig. 1) displays slightly more power (57%) to detect the imprinting effect of a QTL at the 5% significance level than the pedigree without a structure (53%).

Scenario 2

This scenario attempts to examine the effects of different sample sizes and allocation patterns on the parameter estimation of *i*QTL. The results are not shown but summarized as follows. (1) The power to detect an *i*QTL can be increased when an increasing sample size is used. A given size of samples may have different power for *i*QTL detection when samples have different patterns of allocation among families. (2) Equal allocation among different families can increase the power by about one-third relative to unequal allocations as used in the canine pedigree. (3) More families with a smaller size tend to be more powerful than fewer families with a larger size.

Scenario 3

In this scenario, we carried out a reciprocal simulation designed to investigate how well the imprinting model estimates

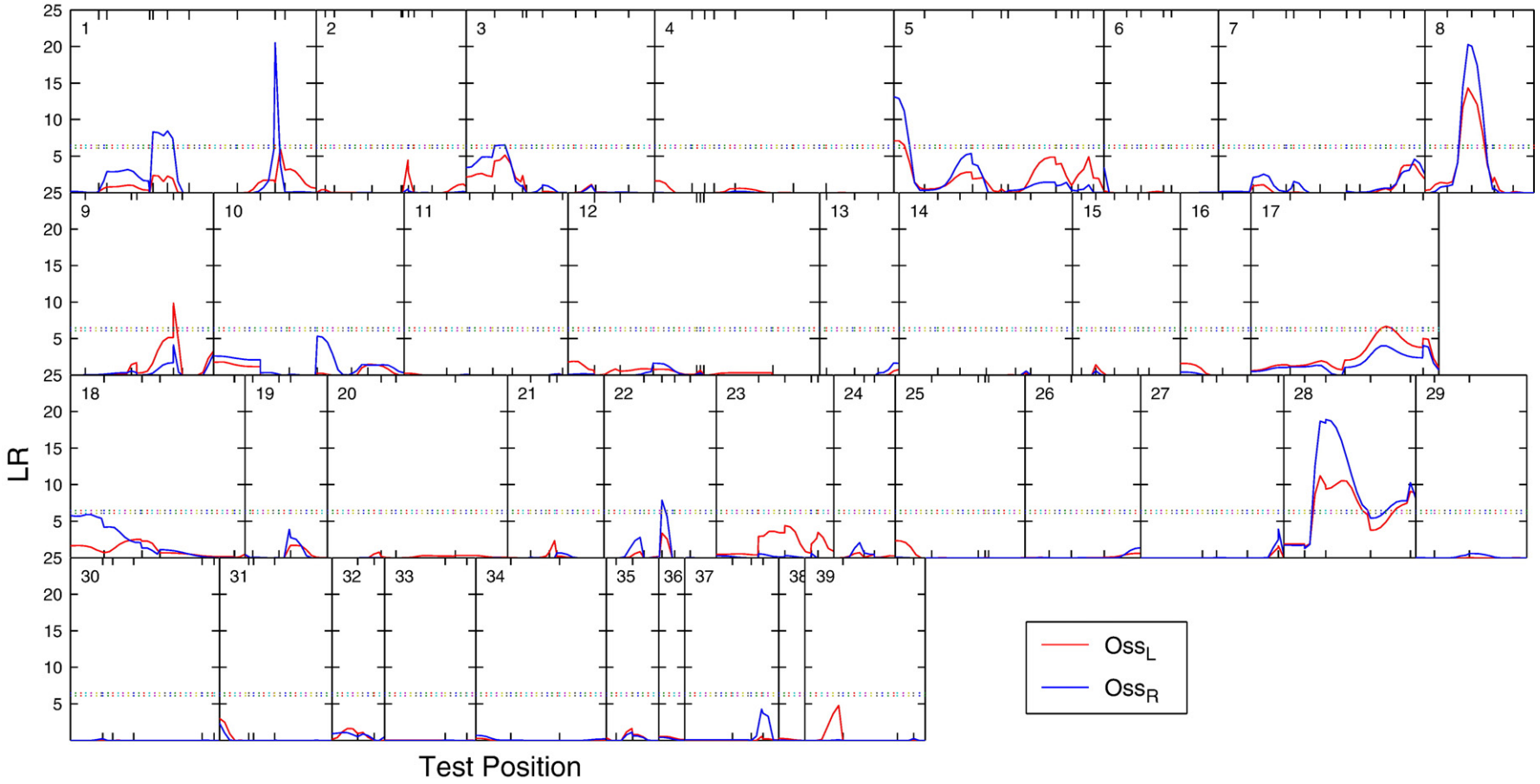

Fig. 2. The profiles of the log_likelihood ratios (LR) calculated from the imprinting model under Hypothesis (7) for OSS measured at the left (OSS_L) and right (OSS_R) canine hip across the entire genome from chromosome 1 to 39 using the linkage map constructed from microsatellite markers. The horizonal line indicates the genome_wide critical threshold at the 1% significance level determined from permutation tests.

Table 2
The MLEs of the QTL position, genetic variances due to maternally (σ^2_{aM}) and paternally derived alleles (σ^2_{aP}), polygenic variance (σ^2_g), and residual variance (σ^2_e) obtained from the imprinting model under two different simulation strategies for independent and related families

	Position (cM)	μ	σ^2_{aM}	σ^2_{aP}	σ^2_g	σ^2_e	Power (%)
Given value	4	10.71	0.30	5.00	0.15	1.87	
Simulation strategy 1: independent families							
MLE	5.08 (1.33)	10.74 (0.06)	0.34 (0.08)	4.63 (0.39)	0.14 (0.04)	1.49 (0.42)	53
Simulation strategy 2: related families							
MLE	4.96 (1.08)	10.69 (0.06)	0.24 (0.07)	4.85 (0.17)	0.15 (0.04)	1.64 (0.25)	57

The numbers in parentheses are the square roots of the mean square errors of the MLEs. Power(%) is calculated as the percentage of the number of simulations in which the imprinted effect of an *i*QTL is detected over the total number of simulations.

the QTL that follows a Mendelian pattern and how poorly the traditional Mendelian model estimates an *i*QTL. The data were simulated under the Mendelian model ($\sigma^2_{aM}=\sigma^2_{aP}=\sigma^2_a$), but analyzed by the imprinting model ($\sigma^2_{aM}\neq\sigma^2_{aP}$). As shown in Table 3, the imprinting model can estimate the genetic variance of a Mendelian QTL well. There is a low Type I error for the detection of the *i*QTL from the imprinting model. But if the data containing an *i*QTL are analyzed by the Mendelian model, the imprinting variance of the QTL will be poorly estimated with a large sampling error. As expected, the Mendelian model has no power to detect an *i*QTL. The results from the reciprocal design suggest that the imprinting model covers the Mendelian model and, thus, can be safely used for any mapping data.

Discussion

The search for genes or QTL that control multifactorial traits, such as complex diseases and developmental disorders, has been a vital area in genetic research over the past 15 years [9,11,23–25]. However, only rare studies report detection of *i*QTL partially because no statistical tool is available for *i*QTL mapping (but see [11,25]). The recent appreciation of gene imprinting [5–8] leads to a new area for epigenetic study that violates traditional Mendelian inheritance since, with imprinted genes, the offspring expresses the trait from only one parent. Gene imprinting provides a new avenue for discovering certain important genes [1–3,26,27].

In this article, we have presented a random-effect model to search for the existence and distribution of *i*QTL throughout the entire genome in a structured outbred pedigree. This model is based on IBD sharing to characterize the relatedness among different members in the pedigree. An *i*QTL is defined as a QTL at which both maternal and paternal alleles are present, but only one allele will be expressed, with the other remaining inactive. Unlike traditional interval mapping models based on Mendelian inheritance [24], the imprinting model allows for the evaluation of the differences between the expression of maternally and paternally inherited alleles at *i*QTL that are identical by descent among siblings in the pedigree.

The model has been used to map *i*QTL for CHD in a structured canine pedigree. As a developmental orthopedic disease, CHD happens at a particular stage of dog development. Some dogs show clear clinical signs of hip dysplasia at a very young age, before arthritis sets in, whereas for many dogs, the symptoms will not be obvious until severe, crippling arthritis has developed. The age at detection of OSS has been used as an important index for the severity of hip dysplasia. A total of 4 chromosomes were observed by our random imprinting model to harbor QTL for OSS at the 1% genome-wide significance level. Some of these QTL are specific to the right metrics of CHD, whereas the others govern the bilateral developmental asymmetry of hip dysplasia. In a companion study of the same pedigree, a fixed-effect model-based internal mapping identified 12 chromosomes that harbor QTL for three different dysplastic traits of canine hips, such as the distraction index, the dorsolateral subluxation score, and the Norberg angle [20].

Table 3
Reciprocal simulation design: parameter estimates and power of the imprinting model for analyzing the Mendelian data and of the Mendelian model for analyzing the imprinting data

Mendelian data	Position (cM)	μ	σ^2_a		σ^2_g	σ^2_e	
Model	5	10.71	5		0.15	1.87	Type I error
Imprinting	5.08 (1.95)	10.76 (0.07)	$\hat{\sigma}^2_{aM}$=2.82 (0.26)	$\hat{\sigma}^2_{aP}$=.82 (0.26)	0.18 (0.09)	1.68 (0.22)	0.04
Imprinting data	Position (cM)	μ	σ^2_{aM}	σ^2_{aP}	σ^2_g	σ^2_e	
	5	10.71	6	0	1	1.87	Type II error
Mendelian	5.08 (1.33)	10.69 (0.05)	$\hat{\sigma}^2_a$=5.63 (0.66)	0.91 (0.17)	2.01 (0.19)		100

The numbers in the parentheses are the square roots of the mean square errors of the MLEs. Type I error (false positive rate) is defined as the rate of *i*QTL detection from the Mendelian model_based simulated data, whereas Type II error (false negative rate) is defined as the rate of *i* QTL that cannot be detected from the imprinting model_based simulated data.

Other studies have also detected significant QTL that affect different developmental aspects of hip dysplasia [23,28]. In a Portuguese water dog population derived from a small group of founders, Lark and colleagues identified two QTL on chromosome 1 that are associated with subluxation of the hip joint, as measured by the Norberg angle—a quantitative radiographic measure of laxity [23]. They further identified a QTL contributing 16% of variance to hip osteoarthritis secondary to hip dysplasia on chromosome 3 in the same population [28]. Some of the detected QTL may represent regions of the genome containing regulatory genes [29] for the control of CHD and its developmental asymmetry. They may be responsible for the rapid evolution of various canine breeds by selection [30].

Our model has been able to identify *i*QTL that play an important role in affecting the age of emergence of hip dysplasia. In several earlier studies of pig genetics [5–7,9], *i*QTL have been observed to affect body composition and heart size in outbred crosses of pigs. The imprinting regulation of *i*QTL may be important for the expression of a number of egg traits in chickens [12] and of muscular hypertrophy in sheep [31]. Some of these detected *i*QTL mapped to the gene for insulin-like growth factor 2 (*IGF2*) and the cell receptor for *IGF2* (*IGF2R*), suggesting the biological ubiquity of imprinted genes in developmental regulation. Comparative genomic analyses of *i*QTL regions among dogs and other mammals such as humans, mice, and pigs will gain insight into the evolution of imprinting genetic effects and their roles in trait and disease formation.

Previous studies have found that imprinted genes are not distributed uniformly throughout the mammalian genome, but tend to cluster together [32,33]. One of the largest clusters is found at the distal end of mouse chromosome 7 and at the proximal end of human chromosome 11*p*15.5 [34]. In the present study, *i*QTL were detected on special chromosomes. Understanding of the distribution of *i*QTL will help to narrow the search interval for important imprinted genes by linkage disequilibrium and haplotype sharing [35–37]. The code to fit the imprinting QTL can be requested from the corresponding author (rwu@stat.ufl.edu).

Materials and methods

The pedigree

A canine pedigree was developed to map QTL responsible for CHD using molecular markers. Seven founding greyhounds and six founding Labrador retrievers were intercrossed, followed by backcrossing F_1's to the greyhounds and Labrador retrievers and intercrossing the F_1's. A series of subsequent intercrosses among the progeny at different generation levels led to a complex network pedigree structure (Fig. 1), which maximized phenotypic ranges in CHD-related quantitative traits and the chance to detect segregating QTL [19,20]. A total of 148 dogs from this outbred population were genotyped for 240 microsatellite markers located on 38 pairs of autosomes and 1 pair of sex chromosomes [21,38]. A linkage map of the canine genome constructed from these markers displays a good coverage of each chromosome. The distances between adjacent markers were estimated in centimorgans for the linkage map [21]. Age at detection of OSS was measured for each of the dog studied at its left and right sides, one of the important criteria for evaluating CHD.

Regression model

Consider multiple related families each with a different number of sibs. A quantitative trait, y, is measured for each sib within each family. The phenotypic value of sib j ($j=1,\ldots,m_i$) within family i ($i=1,\ldots,n$) is expressed as a linear function of K QTL and other fixed covariates, i.e.,

$$y_{ij} = \mu + \sum_{k=1}^{K} \alpha_{ijk} + \sum_{l=1}^{L} \beta_l X_{ijl} + e_{ij}, \tag{1}$$

where μ is the grand mean; α_{ijk} is the effect of the kth QTL expressed in sib j from family i; β_l is the effect of the lth covariate, such as sex or age, which is assumed to be uncorrelated with genetic and environmental errors; X_{ijl} is the variable indicating the lth covariate for sib j from family i; and e_{ij} represents a random environmental error term. The total sample size is $N=\sum_{i=1}^{n} m_i$.

Suppose there is a QTL of interest with an additive effect on the trait. Thus, such a one-QTL model can be written as

$$y_{ij} = \mu + a_{ij} + g_{ij} + \sum_{l=1}^{L} \beta_l X_{ijl} + e_{ij}, \tag{2}$$

where $a_{ij} \sim \mathcal{N}(0, \sigma_a^2)$ is the additive genetic effect; $g_{ij} \sim \mathcal{N}(0, \sigma_g^2)$ is the polygenic additive effect that reflects the effects of unlinked genes or other familial influences, including environmental factors shared by families (excluding the hypothesized QTL); and $e_{ij} \sim \mathcal{N}(0, \sigma_e^2)$ is the environmental error.

Assuming that a_{ij}, g_{ij}, and e_{ij} are uncorrelated random variables each with expectation 0, the total variance for a single observation (y_{ij}) becomes

$$\mathrm{var}(y_{ij}) = \sigma_a^2 + \sigma_d^2 + \sigma_e^2.$$

The covariance between two sibs is

$$\mathrm{cov}(y_{ij}, y_{ij'}) = \pi_{ia}\sigma_a^2 + \phi_{ig}\sigma_g^2,$$

where π_{ia} is the proportion of IBD alleles shared by family members j and j', and ϕ_{ig} is the expected proportion of alleles shared IBD (0.5 for sibling pairs). Therefore, the total variance–covariance matrix for y is given by

$$\boldsymbol{\Sigma} = \boldsymbol{\Pi}_a\sigma_a^2 + \boldsymbol{\Phi}_g\sigma_g^2 + \mathbf{I}\sigma_e^2, \tag{3}$$

where $\boldsymbol{\Pi}_a$ is the matrix of the proportion of marker alleles shared IBD, $\boldsymbol{\Phi}_g$ is the matrix of the expected proportion of alleles shared IBD, and $\mathbf{I}$ is the identity matrix.

To accommodate parent-of-origin effects, the monogenic component of variance can be partitioned into (1) a component that reflects the influence of the QTL carried on the maternally derived chromosome ($\sigma_{a\mathrm{M}}^2$) and (2) a component that reflects the influence of the locus carried on the paternally derived chromosome ($\sigma_{a\mathrm{P}}^2$) [25]. The phenotypic variance–covariance matrix then becomes

$$\boldsymbol{\Sigma} = \boldsymbol{\Pi}_{\mathrm{M}}\sigma_{a\mathrm{M}}^2 + \boldsymbol{\Pi}_{\mathrm{P}}\sigma_{a\mathrm{P}}^2 + \boldsymbol{\Phi}_g\sigma_g^2 + \mathbf{I}\sigma_e^2, \tag{4}$$

where $\boldsymbol{\Pi}_{a\mathrm{M}}$ is the matrix of the proportion of marker alleles shared IBD that are derived from the maternal parent, $\boldsymbol{\Pi}_{a\mathrm{P}}$ is the matrix of the proportion of alleles shared IBD that are derived from the paternal parent, and $\boldsymbol{\Phi}_g$ and $\mathbf{I}$ are defined as above.

When the putative QTL is located on the marker position, it is easy to get the IBD matrix. However, when it is located somewhere between two flanking markers, we need to incorporate the method for determining the IBD matrices, as developed by Fulker and Cardon [39]. The estimation equation for $\boldsymbol{\Pi}_{\mathrm{M}}$ and $\boldsymbol{\Pi}_{\mathrm{P}}$ is given by

$$\hat{\boldsymbol{\Pi}}_{\mathrm{M}} = a + b_1\boldsymbol{\Pi}_{\mathrm{M}1} + b_2\boldsymbol{\Pi}_{\mathrm{M}2},$$

$$\hat{\boldsymbol{\Pi}}_{\mathrm{P}} = a + b_1\boldsymbol{\Pi}_{\mathrm{P}1} + b_2\boldsymbol{\Pi}_{\mathrm{P}2},$$

where $\mathbf{\Pi}_{M1}$, $\mathbf{\Pi}_{M2}$ and $\mathbf{\Pi}_{P1}$, $\mathbf{\Pi}_{P2}$ are the IBD values of maternally and paternally derived alleles, respectively, for the two flanking markers. Fulker and Cardon [39] showed that

$$b_1 = [(1-2r_1)^2 - (1-2r_2)^2(1-2r)^2]/[1-(1-2r)^4],$$
$$b_2 = [(1-2r_2)^2 - (1-2r_1)^2(1-2r)^2]/[1-(1-2r)^4],$$
$$a = (1-b_1-b_2)/2,$$

where r, r_1, and r_2 are the recombination fractions between two linked markers, between the left marker and the QTL, and between the QTL and the right marker, respectively.

Parameter estimation

Under the assumption of multivariate normality, the likelihood function of all phenotypic data (**y**) and flanking markers (**M**) is given by

$$L(\mathbf{\Omega}|\mathbf{y},\mathbf{M}) = (2\pi)^{\frac{N}{2}}|\mathbf{\Sigma}|^{\frac{1}{2}}\exp\left[-\frac{1}{2}\left(\mathbf{Z}^{\mathrm{T}}\mathbf{\Sigma}^{-1}\mathbf{Z}\right)\right], \quad (5)$$

with $\mathbf{Z} = \mathbf{y} - \mathbf{1}\mu - \mathbf{X}\beta^{\mathrm{T}}$, where $\mathbf{y} = \{y_{im_i}\}_{i=1}^{n}$ is an $(N \times 1)$ vector of all phenotypes, $\mathbf{1}$ is an $(N \times 1)$ vector with all entries equal to 1, $\beta = (\beta_1,\ldots,\beta_L)$ is the L-dimensional covariate effect, and $\mathbf{X} = \{X_{im_iL}\}_{i=1}^{n}$ is the design matrix. We can rewrite

$$\mathbf{\Sigma} = \sigma_e^2(\mathbf{\Pi}_{M\gamma M} + \mathbf{\Pi}_{P\gamma P} + \mathbf{I}),$$

where $\gamma_M = \sigma_M^2/\sigma_e^2$ and $\gamma_P = \sigma_P^2/\sigma_e^2$, and define

$$\mathbf{H} = \mathbf{\Pi}_{M\gamma M} + \mathbf{\Pi}_{P\gamma P} + \mathbf{I};$$

hence $\mathbf{\Sigma} = \sigma_e^2\mathbf{H}$. Then, the likelihood function becomes

$$L(\mathbf{\Omega}|y,\mathbf{M}) = (2\pi)^{-\frac{N}{2}}\sigma_e^{-N}|\mathbf{H}|^{-\frac{1}{2}}\exp\left[-\frac{1}{2\sigma_e^2}\left(\mathbf{Z}^{\mathrm{T}}\mathbf{H}^{-1}\mathbf{Z}\right)\right]. \quad (6)$$

By taking the derivative of the log-likelihood function with respect to μ, β_l, and σ_e^2, we obtain their MLEs as

$$\hat{\mu} = (\mathbf{1}^{\mathrm{T}}\mathbf{H}^{-1}\mathbf{1})^{-1}(\mathbf{1}^{\mathrm{T}}\mathbf{H}^{-1}\mathbf{y}),$$
$$\hat{\beta} = (\mathbf{X}^{\mathrm{T}}\mathbf{H}^{-1}\mathbf{X})^{-1}(\mathbf{X}^{\mathrm{T}}\mathbf{H}^{-1}\mathbf{y}),$$

and

$$\hat{\sigma}_e^2 = \frac{1}{N}\left(\mathbf{y} - \mathbf{1}\hat{\mu} - \mathbf{X}\hat{\beta}^{\mathrm{T}}\right)^{\mathrm{T}}\mathbf{H}^{-1}\left(\mathbf{y} - \mathbf{1}\hat{\mu} - \mathbf{X}\hat{\beta}^{\mathrm{T}}\right).$$

By plugging $\hat{\mu}$, $\hat{\beta}$, and $\hat{\sigma}_e^2$ into the log-likelihood function, the MLEs of γ_M and γ_P are estimated using the simplex algorithm.

Hypothesis tests

After the parameters are estimated, we test two hypotheses regarding the existence of QTL and iQTL, respectively. Whether there is a QTL can be tested by formulating the following hypotheses:

$$H_0 : \gamma_M = \gamma_P = 0$$
$$H_1 : \text{At least one of } \gamma's \text{ is not equal to zero.} \quad (7)$$

The likelihoods under the null ($L_0(\tilde{\Omega}|\mathbf{y})$) and alternative hypotheses ($L_1(\hat{\Omega}|\mathbf{y},\mathbf{M})$) are calculated, with which the log-likelihood ratio is calculated by

$$LR = -2[\ln L_0(\tilde{\Omega}|\mathbf{y}) - \ln L_1(\hat{\Omega}|\mathbf{y},\mathbf{M})], \quad (8)$$

where $\tilde{\Omega}$ and $\hat{\Omega}$ are the MLEs of parameters under H_0 and H_1, with the former not affected by marker genotypes. The critical value for the declaration of the existence of QTL can be empirically determined by permutation tests.

To test whether the detected QTL is imprinted, we formulate the Mendelian model as the null hypothesis, i.e.,

$$H_0 : \gamma_M = \gamma_P$$
$$H_1 : \gamma_M \neq \gamma_P. \quad (9)$$

Whether the *i*QTL is completely maternally or paternally imprinted can be tested using the following hypotheses:

$$H_0 : \gamma_M = 0$$
$$H_1 : \gamma_M \neq 0, \quad (10)$$
$$H_0 : \gamma_P = 0$$
$$H_1 : \gamma_P \neq 0. \quad (11)$$

Hypothesis (9) is related to partial imprinting, with the direction depending on $\gamma_M > \gamma_P$ or $\gamma_M < \gamma_P$. Hypotheses (10) and (11) are associated with the complete imprinting of the QTL allele inherited from the maternal and paternal parents, respectively. The MLEs of parameters under H_0 of Hypotheses (9–11) are obtained with the algorithm as described above, but with constraints posed by the corresponding H_0. The log-likelihood ratio (LR_I) for the detection of *i*QTL can be calculated accordingly. The determination of the critical thresholds for all these hypotheses was based on simulation studies.

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