Available online at www.sciencedirect.com
ScienceDirect
Genomics 90 (2007) 276–284
GENOMICS
www.elsevier.com/locate/ygeno

Method

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

Tian Liu [a], Rory J. Todhunter [b], Song Wu [a], Wei Hou [c], Raluca Mateescu [b], Zhiwu Zhang [d], Nancy I. Burton-Wurster [e], Gregory M. Acland [e], George Lust [e], Rongling Wu [a,*]

[a] Department of Statistics, University of Florida, Gainesville, FL 32611, USA
[b] Department of Clinical Sciences, Cornell University, Ithaca, NY 14853, USA
[c] Department of Epidemiology and Health Policy Research, University of Florida, Gainesville, FL 32611, USA
[d] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853, USA
[e] James A. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

Received 16 September 2006; accepted 6 April 2007
Available online 24 May 2007

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

* Corresponding author. Fax: +1 352 392 8555.
E-mail address: rwu@stat.ufl.edu (R. Wu).

0888-7543/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygeno.2007.04.004

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