Quantitative Trait Loci Determining Weight Reduction of Testes and Pituitary by Diethylstilbesterol in LEXF and FXLE Recombinant Inbred Strain Rats

Masayoshi TACHIBANA1)*, Lingmin LU2, 3), Hiroshi HIAI2, 4), Atsushi TAMURA1), Yoshibumi MATSUSHIMA1), and Hayase SHISA1)

1)Saitama Cancer Center, 818 Komuro, Ina, Saitama 362-0806, 2)Department of Pathology and Biology of Diseases, Kyoto University Graduate School of Medicine, Yoshida-Konoe-Cho, Sakyoku, Kyoto 606-8501, Japan, 3)McMaster University, Hamilton, Canada, and 4)Shiga Medical Center for Adults, 5–4–30 Moriyama, Moriyama City, Shiga 524-8524, Japan

Abstract: Increasing exposure to environmental endocrine disruptor, xeno-estrogen, is a serious hazard to male reproductive activity. To explore possible genetic control in susceptibility to xeno-estrogen, the weight reduction of testes induced by the continuous administration of a synthetic estrogen, diethylstilbesterol, were investigated by quantitative trait analysis in LEXF and FXLE recombinant inbred strain rats, consisting of 21 independent strains, 9 of their substrains, parental F344/Stm and LE/Stm strains, and (F344 x LE)F1. For the weight of testes, one highly significant quantitative trait locus (QTL) and one significant QTL were mapped on chromosomes 7 and 1, respectively. The QTL on chromosome 7 is closely associated with c-myc. Pituitary weight and serum prolactin were also variable among recombinant inbred strains, but no QTL was detected for them in this study.

Key words: diethystilbesterol, LEXF and FXLE rat, QTL, recombinant inbred strain, testis

Introduction

Recent declines in male reproductive health, including a reduction in sperm count and testicular weight, and an increase in testicular cancer, hypospadias, and cryptorchism, are being attributed to an environmental endocrine disrupter, xeno-estrogen [8, 10]; however, other factors should be considered as well. Geographic differences in the incidence of reproductive anomalies have been noted in various countries and among races, and genetic factors and/or differences in lifestyle and environment are suggested to be involved in these differences (see Editorial, The Lancet, Vol. 345, April 1995). In an effort to determine whether and how genetic factors are involved, we undertook the localization of the possible gene responsible for testicular weight reduction in xeno-estrogen exposure. For this purpose, we employed quantitative trait locus (QTL) mapping.
using LEXF and FXLE recombinant inbred (RI) rats given diethylstilbesterol (DES), a synthetic estrogen with strong xeno-estrogenic activity, to reduce testis weight [7]. We identified a QTL significantly associated with DES-induced reduction of testis weight on chromosome 7, close to a marker locus D7Mit4, and another QTL on chromosome 1. In this report, QTL mapping data is presented, and a possible candidate for this QTL is discussed.

Materials and Methods

RI strain rats

We previously established a set of RI strains, named LEXF RI rats, from (LE/Stm × F344)F2 [11]. Later, additional recombinant strains FXLE were generated from (F344 × LE/Stm)F2. It was assumed that the LEXF and FXLE rats shared the same gene pool. In this study, we used the following strains of rats: LEXF-1A, B, C, LEXF-2A, B, C, LEXF-3, LEXF-4, LEXF-5, LEXF-6A, LEXF-7A, B, C, LEXF-8A, C, D, LEXF-9, LEXF-10A, C, LEXF-11, FXLE-12, FXLE-13, FXLE-17, FXLE-18, FXLE-20, FXLE-22, FXLE-23, FXLE-24, FXLE-25, FXLE-26, F344, LE/Stm, and (F344 × LE/Stm)F1. These 33 strains included 21 independent RI strains, 9 substrains, two parental strains, and their F1 hybrid. The substrains of independent RI strains, as shown in symbols A-D, were also used. These substrains were generated to analyze the coat color of the loci during the establishment of the RI strains [11].

DES administration and phenotype

Four-week-old male rats were subcutaneously implanted with a polystyrene tube (10 mm long and 2 mm in inner diameter (Kaneka Medics, Tokyo, Japan), containing ~17 mg of DES) in the dorsal region. Twelve weeks later, the animals were sacrificed under ether anesthesia, and both testes were removed and weighed together. At the same time, the weight of the pituitary gland and the serum prolactin level were determined. A solid-phase radioimmunoassay was used to measure the serum prolactin level. From each strain, 8.5 ± 4.9 (12 to 5) rats were used for phenotype determination.

Genetic analysis

The strain distribution pattern (SDP) tables for the genotype of genome-wide-distributed marker loci have been published elsewhere [4, 11]. The SDP consisted of a total of 153 marker loci, i.e., 142 microsatellites and 11 biochemical genetic markers. The latest expanded version (version 3) is available at the home page of the National Bioresource Project for the Rat in Japan (http://www.anim.med.kyoto-u.ac.jp/NBR/Rat_Links.htm). Interval QTL mapping analysis was performed with Map Manager/QTb29 software [6] (http://www.mapmanager.org/mmQTX.html). LOD (logarithm of odds) values were calculated to evaluate significance of linkage. The minimum LOD value indicating significance was 2.8 and suggestive linkage, 1.7.

Results

After DES treatment, the weight of the testes remarkably decreased. For instance, the weight of testes in untreated LE/Stm rats was 3189 ± 288 mg, and in DES-treated rats, 147 ± 8 mg. In untreated F344, it was 2716 ± 212 mg and in DES-treated F344, 200 ± 12 mg. Among RI strains, there was little correlation in testes weight between pre- and post-treatment (r<0.31). As shown in Fig. 1, the weight was variable from one strain to another after DES treatment. Analysis with Map manager/QTb29 revealed a highly significant QTL peak on chromosome 7, showing a LOD score of 3.28 between D7Mit5 and D7Mit4 (Fig. 2A). The percentage of the phenotype value explained at D7Mit4 was 36%. A protooncogene, c-myc, was mapped between D7Mit5 and D7Mit4. Another significant linkage was found on chromosome 1 with an LOD score of 2.82 at the closest marker locus Spe1 (Fig. 2B). The percentage of the phenotype value explained at Spe1 was 31%. Except for these two QTLs, no other QTL, even at the suggestive level, was detected in the present study.

Treatment with DES significantly increased the weight of the pituitary gland. Figure 3A shows the pituitary weight of DES-treated LEXF RI rats, and Fig. 3B, the serum prolactin level. There was a highly significant correlation between the pituitary weight and the serum prolactin level (r=0.987) (Fig. 4). Pituitary weight and serum prolactin level were both variable among RI strains, however, QTL analyses did not detect any significant or suggestive linkage for either trait.
The present study revealed that genetic factors are indeed involved in the DES-induced reduction of testis weight. The genetic trait was polygenic in nature. The QTL peak showing the highest LOD score was closely mapped to the c-myc gene and its activator genes, Pvt1 and Mlvi4. A number of studies have reported that c-myc plays an important role in spermatogenesis. Increased c-myc mRNA is observed in regenerating testes after X-irradiation [2]. Overexpression of c-myc induces apoptosis at the prophase of meiosis of rat primary spermatocytes [3]. It is possible; therefore, that genetic polymorphism in c-myc may be associated with the susceptibility to endocrine disruption of the testes. On the other hand, we did not detect any significant or suggestive QTLs in the reduction in the pituitary weight and serum prolactin level. This suggests that these two traits may not be under genetic control, or alternatively, the number of RI strains used in this study, or the number of marker loci, is not sufficient to analyze complex traits.

Estrogen-induced pituitary tumorigenesis in the rat is under genetic control [12, 13]. Wendell and Gorski [12] mapped 5 QTL affecting pituitary tumorigenesis on chro-
mosomes 2, 3, 5, 9 and 14 in (F344 × BN)F2 rats.  More recently, Shull et al. [14] identified 6 QTLs in (ACI × COP)F2 rats, one locus each on chromosome 6 and 9, and two loci each on chromosomes 3 and 1.  Although the strain combination and quantitative phenotype observed were different from our study, further investigation is indicated as there was a remarkable strain variation among RI strains.  At the very least, the present study provides evidence that the effect of DES on endocrine organs is differentially controlled by host genes.

RI strains have been demonstrated to be a useful tool in the first step of mapping complicated genetic traits [1, 5, 9].  In this study, we used 30 LEXF and FXLE RI strains and their parental strains.  Genetic analysis was performed by simply processing phenotype values and published SDP data with QTL mapping software.  This approach greatly saved time, labor, and cost when compared with conventional intercrossing experiments.  At present, the number of RI strains is increasing, and their SDP data is expanding.  For QTL mapping with

Fig. 3.  A: Weight of pituitary gland of each RI strain given diethylstilbestrol.  B: Serum prolactin level after diethylstilbestrol administration.  Vertical bars represent the average and standard deviation.
RI strains, the bigger the difference in phenotypic values between parental strains is, the greater the possibility of identification of QTL is. However, for complicated traits, this is not a rule, since combinations of genes affecting phenotypic values, positively or negatively, finally determine the phenotypic values.

The LEXF and FXLE RI strains rats are now deposited in the National Rat Bioresource Project at Kyoto University and information on their latest genetic and phenotypic profile is available on their home page (http://www.anim.med.kyoto-u.ac.jp/NBR/Rat_Links.htm).

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