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Age-dependent and tissue-specific structural changes in the C57BL/6J mouse genome

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ABSTRACT

We tested the hypothesis that structural changes in the genome parallel age- and organ-specific phenotypes in conjunction with the differential transposition activities of retroelements. The genomes of the liver from C57BL/6J mice were larger than other organs, coinciding with an increase in genomic copies of certain retroelements. In addition, there were differential increments in the genome size of the liver with increasing age, which peaked at 5 weeks. The findings that the genome structure of an individual is variable depending on age and organ type in association with the transposition of retroelements may have broad implications in understanding biologic phenomena.

Introduction

The entire set of genes/protein coding sequences constitutes ~3% of the human and mouse genomes. In contrast, the vast majority (~45%) of the genome is composed of both characterized and uncharacterized families of retroelements (Venter et al., 2001; Waterston et al., 2002). Functional characterization of genes, including their polymorphic variants, has been the core element of biomedical studies for the last several decades (Schuler et al., 1996; Venter et al., 2001). Substantial progress has been made in understanding the mechanisms underlying both normal and disease phenotypes by studying the function of individual genes as well as interaction among different gene products (Arisi et al., 2011; Xia et al., 2007). However, thus far, it was unsuccessful to fully explain a number of phenotypes, both normal (e.g., aging) and disease (e.g., tumor), solely on the basis of gene functions.

The human and mouse genomes contain heterogeneous families of retroelements, which include long interspersed nuclear elements (LINEs), short interspersed nuclear elements (SINEs), and endogenous retroviruses (ERVs) (Venter et al., 2001; Waterston et al., 2002). The results from our recent studies have demonstrated that stress signals elicited from injury and/or infection activate specific groups of ERVs in an organ/cell-type specific manner (Cho et al., 2008; Kwon et al., 2009; Lee et al., 2007). In addition, lipopolysaccharide stimulation of mouse primary lymphocytes resulted in an increase in the production of certain ERV virions, which retain the potential to infect neighboring cells followed by random integration of new proviral copies into the genome (Kwon et al., 2011).

Currently, most biologic processes, such as aging and tumorigenesis, have been explained by common polymorphisms of genes found among different individuals. We speculate that there are potential roles for uncommon variations, which are uniquely introduced into the personal genome during the lifespan of an individual, in phenotype determination. In this study, we tested the hypothesis that genomic structural changes, a form of uncommon variation, parallel age-dependent and organ-specific phenotypes in an individual in conjunction with the differential transposition activities of retroelements.

Materials and methods

Animals

Eight different age groups (~2 to ~29 weeks) of C57BL/6J mice (♀) were purchased from the Jackson Laboratory (West Sacramento, CA). In addition, three older age groups (40 weeks [♂], 60 weeks [♂], and 77 weeks [♀]) of C57BL/6J were obtained from Dr. David Pleasure of the University of California, Davis. The protocol was approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis. Three to five mice from each age group were sacrificed by CO_2 inhalation and the collected tissues were snap-frozen in liquid nitrogen.

Genomic DNA preparation

Genomic DNAs from different tissues were isolated using DNeasy Tissue kit (Qiagen, Valencia, CA) with an RNase A (Qiagen) treatment option. Each DNA sample was normalized to 20 ng/μl based on spectrophotometer (Beckman Coulter, Brea, CA) readings at 260 nm.

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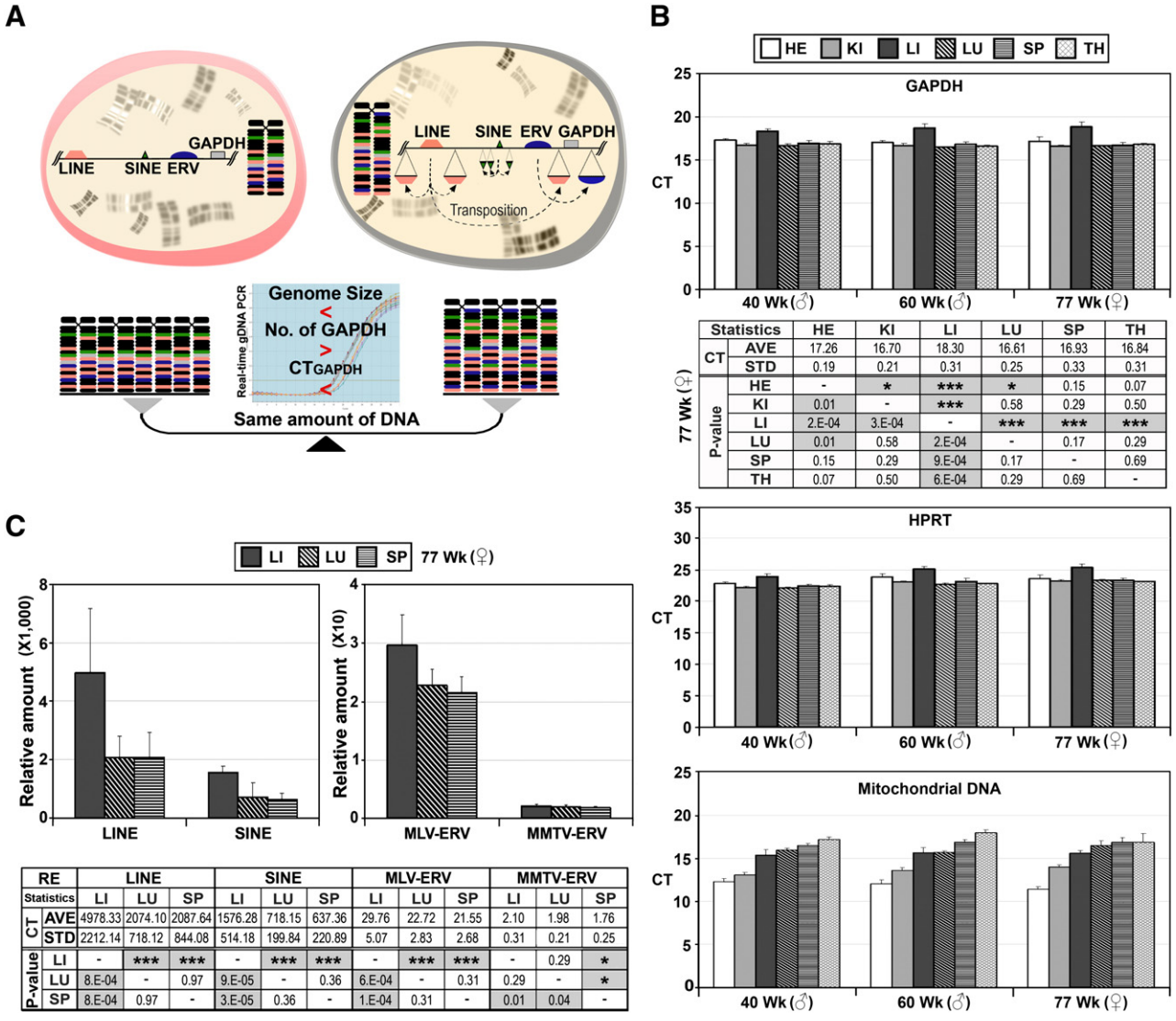
Control genomic DNA was prepared from a mixture of mouse tissues (liver, lung, kidney and spleen) and aliquoted into six tubes for both the normalization process and polymerase chain reaction (PCR). Each aliquot of the control genomic DNA was independently normalized to 20 ng/μl.

Genomic DNA real-time PCR

The sequence and PCR condition for each primer set are shown in Supplementary Table 1. The efficiency and specificity of each primer set were evaluated using six different dilutions (25, 2.5, 1, 0.1, 0.01 and 0.001 ng) of the control genomic DNA. The slopes of the standard curve were in the range of -3.32 ± 0.2. Real-time PCR was performed using a MX3005P instrument (Stratagene, Santa Clara, CA) with a reagent kit (Brilliant SYBR Green QPCR Master Mix) from Agilent (Santa Clara, CA) and 2.5 ng (for SINE) or 25 ng (for all the other targets) of each genomic DNA in triplicate. Potential run-to-run variations from the individual reactions were compensated with the results from the control genomic DNA aliquots, which were included in each PCR run. Details for the real-time PCR conditions are listed in Supplementary Table 1.

Calculation and statistics

All cycle threshold (CT) values were normalized using the CT values of the control DNA in each run. The quantity of each retroelement was calculated as a relative copy number per single copy of hypoxanthine phosphoribosyl transferase (HPRT) gene using a modified delta-delta CT method ($2^{\wedge}(CT_{(HPRT)} - CT_{(retroelement)})$). A two-tailed Student's *t*-test was used to determine the significance of differences in CT values for the comparison of genome size and retroelement copy number within each combination of two tissues. In addition, a



77 Wk(♀)	Statistics	HE	KI	LI	LU	SP	TH
CT	AVE	17.26	16.70	18.30	16.61	16.93	16.84
	STD	0.19	0.21	0.31	0.25	0.33	0.31
P-value	HE	-	*	***	*	0.15	0.07
	KI	0.01	-	***	0.58	0.29	0.50
	LI	2.E-04	3.E-04	-	***	***	***
	LU	0.01	0.58	2.E-04	-	0.17	0.29
	SP	0.15	0.29	9.E-04	0.17	-	0.69
	TH	0.07	0.50	6.E-04	0.29	0.69	-

RE		LINE			SINE			MLV-ERV			MMTV-ERV		
Statistics		LI	LU	SP	LI	LU	SP	LI	LU	SP	LI	LU	SP
CT	AVE	4978.33	2074.10	2087.64	1576.28	718.15	637.36	29.76	22.72	21.55	2.10	1.98	1.76
	STD	2212.14	718.12	844.08	514.18	199.84	220.89	5.07	2.83	2.68	0.31	0.21	0.25
P-value	LI	-	***	***	-	***	***	-	***	***	-	0.29	*
	LU	8.E-04	-	0.97	9.E-05	-	0.36	6.E-04	-	0.31	0.29	-	*
	SP	8.E-04	0.97	-	3.E-05	0.36	-	1.E-04	0.31	-	0.01	0.04	-

Fig. 1. Relatively larger genome size of the liver compared to the heart, kidney, lung, spleen, and thymus. **A.** The schema of how the real-time genomic DNA PCR protocol was developed to measure the relative genome size is illustrated. When two different genomic DNAs of the same amount/weight are compared, a lower GAPDH copy number, which is reflected in a higher CT value from real-time PCR, indicates a larger genome size. **B.** Six different tissues (heart [HE], kidney [KI], liver [LI], lung [LU], spleen [SP], and thymus [TH]) from three age groups of C57BL/6J mice were subjected to genomic DNA real-time PCR analyses for GAPDH, HPRT, and mitochondrial DNA. The GAPDH CT values of the liver genomic DNA were higher than those from the other tissues in all three age groups, indicating that the liver's genome size is larger than the others. HPRT and mitochondrial DNA served as controls for an additional static genomic element and non-genomic DNA, respectively. Wk (week), AVE (average), STD (standard deviation), CT (cycle threshold), $^{*}P<0.05$, $^{***}P<0.001$. **C.** The contribution of four retroelement subfamilies (LINE, SINE, MLV–ERV, and MMTV-ERV) to the larger genome size in the liver was examined by real-time PCR. The copy numbers of LINEs and SINEs were higher in the liver than in the lung and spleen, which paralleled the greater number of MLV–ERV and MMTV-ERV copies in the liver. Wk (week), RE (retroelement), AVE (average), STD (standard deviation-error bar), CT (cycle threshold), $^{*}P<0.05$, $^{***}P<0.001$.