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Genomic structure and genetic drift in C57BL/6 congenic metabolic mutant mice

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ABSTRACT

We used a genome-wide single nucleotide polymorphism (SNP) approach to characterize the genomic structures of four representative C57BL/6 (B6) congenic mutant mouse lines to include the A) long-chain acyl-CoA dehydrogenase (*Acadl*), B) melanocortin 3 receptor (*Mc3r*), C) endothelial nitric oxide synthase (*Nos3*), and D) a replacement of mouse apolipoprotein E (*Apoe*) by human apolipoprotein E-2 (*APOE2*). We wanted to evaluate the size and flanking genes of the 129 strain origin mutant allele intervals on the B6 background. Additionally, we wanted to evaluate genetic drift among not only the four mutant lines and their respective B6 origin substrains, but also the drift between two commonly used B6 lines obtained from Jackson Laboratory and Taconic. Overall, we found a range of 129 origin interval sizes in the congenic mutant lines analyzed that ranged from a ~2.8 kb human sequence for *APOE2* embedded in a 129S6 interval to the largest being a ~16 Mb fragment containing the targeted *Nos3* (eNos) gene. Given the range of 129 strain interval sizes, we found 129 strain flanking genes via annotation in genome data bases ranging from one gene both upstream and downstream of the *APOE2* allele to seven genes-upstream and five genes-downstream at the *Nos3* locus. Furthermore, we found fourteen SNP differences between the Jackson Laboratory and Taconic B6 mice. These genetic differences were associated with marked adiposity differences between the two B6 substrains. This study demonstrates both the fidelity and the caveats of using congenic gene targeted mouse models and recognizing the importance of selecting the appropriately matched wild-type control mouse line.

1. Introduction

Congenic mutant mice are widely used in biomedical research. The genetic approach goes back to the pioneering work of George Snell at the Jackson Laboratory as reviewed by Silver [1]. One of several C57BL/6 (B6) substrains is frequently used as the recipient strain in the backcross. Many gene targeted mouse models have been developed into congenic mutant lines by at least a 10 generation backcross to the chosen B6 substrain and then used in experiments with the wild-type (WT) B6 mice as controls. What is often not known, recognized or even considered are the flanking sequences adjacent to the selected mutant alleles from the original donor mouse strain of the targeted embryonic stem cell, often one of several possible 129 mouse strains. Due to our interests in complex metabolic disease and multigene models, we pursued a fine mapping approach to characterize the genomic structure of four different congenic lines as representative examples of the genomic structure and accompanying targeted genes. This analysis was based on using informative single nucleotide polymorphisms (SNPs) that would distinguish the different mouse inbred strains and substrains [2]. We wanted to answer the question, what is the interval size of flanking sequence from an origin strain (e.g., 129) of a gene targeted locus, and how many flanking genes represent the origin strain alleles rather than the B6 alleles as the recipient strain of the congenic mutant line? In this study we characterized the genomic structures of four congenic mutant loci on two different B6 backgrounds to include the Jackson Laboratory C57BL/6J (B6/J) substrain and the Taconic version C57BL/6NTac (B6/Tac) substrain. We demonstrate these genomic structures with four gene targeted example models; A) long-chain acyl-CoA dehydrogenase (gene = *Acadl*; designated LCAD.B6/Tac) [3,4], B) melanocortin 3 receptor (*Mc3r*; MC3R.B6/J) [5], C) endothelial nitric oxide synthase (*Nos3*; eNOS.B6/J) [6], and D) a replacement of mouse *Apoe* by human *APOE2* (APOE2.B6/Tac) [7].

Furthermore, there are many B6 substrains available that have been separated by decades and interbred within individual laboratory colonies or vendor locations allowing for genetic drift to occur, thus all B6 mice are not equivalent. There are striking phenotypic differences

Abbreviations: B6, C57BL/6 strain; B6/J, Jackson Laboratory C57BL/6J substrain; B6/Tac, Taconic version C57BL/6NTac substrain; gene = *Acadl*, protein = LCAD, long-chain acyl-CoA dehydrogenase; gene = *Mc3r*, protein = MC3R, melanocortin 3 receptor; gene = *Nos3*, protein = eNOS, endothelial nitric oxide synthase; gene = *Apoe*, protein = APOE, mouse apolipoprotein E; gene = *APOE-2*, protein = APOE-2, human apolipoprotein E-2; gene = *Nnt*, nicotinamide nucleotide transhydrogenase; SNP, single nucleotide polymorphism; WT, wild-type.

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between B6/J and B6/Tac mice, e.g., adiposity, and these differences are accompanied by genomic variations as surveyed by a genomic wide SNP analysis reported here. Until now, a single locus nicotinamide nucleotide transhydrogenase (*Nnt*) was the most widely recognized genetic variant between B6/J mice and others like B6/Tac [8]. What is the genetic drift between two commonly used B6 substrains (JAX or Tac) used in developing the four congenic mutant strains investigated here? These substrains have been separated more than 60 years. This point is also frequently overlooked, but may be very important when considering the phenotypes being studied and choosing the appropriate WT control B6 line [9]. Thus, via a genome-wide SNP based approach, we evaluated the genetic drift between current B6/J and B6/Tac substrains with their corresponding congenic mutant lines, and the genetic drift between the B6/J and B6/Tac substrains compared directly.

2. Materials and methods

The parental background inbred substrains included C57BL/6J (Jackson Laboratory), C57BL/6NTac (Taconic) and 129S6 (Taconic). The congenic mutant strains included LCAD.B6/Tac [3] (in house colony, backcrossed 10 generations), APOE2.B6/Tac [7] (Taconic, backcrossed 9 generations), MC3R.B6/J [5] (Jackson Laboratory, backcrossed >10 generations) and eNOS.B6/J [6] (Jackson Laboratory, backcrossed 12 generations). Genomic DNA was prepared from tail tip biopsy and was extracted using a Wizard Genomic DNA Purification Kit (Promega). Adiposity was evaluated in B6/J and B6/Tac male mice at 12 weeks of age using a NMR instrument (Bruker Minispec Analyzer). The mice were fed Harlan-TekLad Diet 2019. All animal procedures were approved by the IACUC of the Sanford-Burnham Medical Research Institute.

Initially, we surveyed the *Acadl* locus in the LCAD.B6/Tac congenic line using the Taqman SNP (Life Technologies) individual SNP assays. We used the Jackson Laboratory SNP database to interrogate 10 million base pairs (Mb) upstream and downstream of the *Acadl* gene. Then we compared the SNPs of congenic line against B6/Tac and 129S6 mouse genomic DNA. These experiments proved too cumbersome and expensive to achieve the desired characterization of the mutants, so we genotyped the mouse lines using a SNP array as an alternative approach [10].

For the global SNP analyses of the four B6 congenic lines and the three WT (B6/J, B6/Tac, 129S6) lines, all genomic DNA samples were processed using the Genome-wide Mouse Diversity Array [11,12] (Affymetrix) processing kit, as described in the Affymetrix Genome-Wide SNP Nsp/Sty 6.0 user guide [13]. Results were processed using the mouse genotyping console (Affymetrix) and the data were harvested and filtered to compare all mouse lines. For the ultrafine mapping, only the B6/Tac, LCAD.B6/Tac and 129S6 genomic DNAs were used. A ~2 k upstream region flanking the *Acadl* gene was sequenced using AB 3100 capillary sequencing system (Life Technologies). Those sequences were compared and SNPs were identified using Vector NTI (Life Technologies). SNPs used for genotyping the *Acadl* locus for the LCAD mutant mouse strain were derived by using individual sequencing assays as described [4].

3. Results

3.1. Targeted gene (129 strain) locus and flanking sequences on a B6 background

Our initial approach was to characterize the *Acadl* locus on chromosome 1 in WT B6/Tac mice compared to *Acadl*-targeted mice also on a B6/Tac background. For this analysis, we used SNP data base-derived informative sites, which were interrogated by individual SNP PCR assays. Using this approach we identified a 129 DNA sequence interval of approximately ~6.5 Mb containing *Acadl* (~32 kb). This included ~1.5 Mb upstream and ~5 Mb downstream of 129 genomic sequence flanking the *Acadl* gene locus (Table 1). This defined interval was confirmed (Fig. 1) using a genome-wide SNP-based approach, which screens 623,000 SNPs and has a map resolution of 4.3 kilobases (kb). From this analysis, we identified five genes downstream and four genes upstream of 129 origin (Table 4). We also analyzed three additional mutant lines using this genome-wide SNP approach. We continued this approach to include the targeted locus in the APOE2.B6/Tac mouse line, which similar to the *Acadl* mutation is also on a B6/Tac background (Fig. 1). The APOE2.B6/Tac mouse line contains a replacement of mouse native *Apoe* locus by human *APOE2* exons 2–4. SNP analysis of this line shows that this replacement is located in a very small 129 interval of only ~2.8 kb (Fig. 1) indicating that the APOE2 replacement exons are embedded within the 129 locus are the human *APOE2* exons. In addition, we found the *comm*40 gene to be located downstream while the *Apoc1* gene was located upstream of the targeted locus. However, these genes were located outside the 129 strain interval, i.e. of B6/Tac origin (Table 4).

We also evaluated the targeted mutations for *Mc3r* (Table 2) and *Nos3* (Table 3), which are both on a B6/J background (Fig. 1). In our analysis of the MC3R.B6/J mouse line, we found a 129 sequence interval of ~4 Mb on chromosome 2 containing *Mc3r* (~2.6 kb). This region was characterized by ~3.5 Mb 129 sequence upstream of the *Mc3r* locus (containing five genes), while downstream of the locus spanned ~0.5 Mb (containing four genes). Evaluation of the eNos.B6/J mouse line showed that the *Nos3* locus (~19.7 kb) located on chromosome 5, was contained within an ~8 Mb interval of 129 sequence with 5.0 Mb upstream (containing seven genes) and ~3.0 Mb downstream (containing five genes) (Table 4). Therefore, in the four gene targeted mutants analyzed, we found 129 flanking sequence intervals ranging from ~2.8 kb for *APOE2* to ~8 Mb for *Mc3r*.

3.2. Congenic mutant mouse line compared to parental line B6/J or B6/Tac

Next, we wanted to evaluate the potential genetic drift between the congenic mutant lines B6 background strain genome and the parental line WT B6 mice that may act as WT controls. As shown in Table 1, out of 623,000 SNPs interrogated as a proxy for mutational differences, we found a total of 18 differences between the LCAD.B6/Tac line and the parental line B6/Tac. Of the 18 differences (Table 1), all 18 were located on chromosome 1 and were located within the identified 129 strain sequence interval containing the *Acadl* gene targeted locus indicating no detectable differences at least at the SNP loci scattered throughout the congenic background genome. The APOE2.B6/Tac compared against the parental B6/Tac revealed no differences, which was not surprising given the extremely small targeted region containing 129/human sequences.

The MC3R.B6/J mutant compared to the B6/J demonstrated 9 differences all contained on chromosome 2 and the 129 sequence interval harboring the *Mc3r* mutant allele (Table 2). Likewise, the eNOS.B6/J mutant had 24 SNP differences from the parental B6/J line and all were contained within the relatively larger ~8 Mb 129 sequence interval (Table 3). Consistently among the four mutant lines investigated, the number of SNP variants was proportional with the 129 interval size and all the SNP variants were located within the 129 sequence region flanking the targeted mutant alleles rather than in the B6 background components of the genome.

3.3. SNP variation between B6/J and B6/Tac parental strains

We also wanted to evaluate the SNP variation between the two parental B6 substrains investigated here beyond the known *Nnt* mutation found in B6/J. Part of our interest in this was driven by the drastic differences in phenotype that may exist among B6 substrains. In our analysis of the two parental B6 substrains, we detected marked differences in adiposity. As shown in Fig. 2, among male mice fed