ABSTRACT

Objectives:
1. Evaluate gravity receptor function in different strains of mice using the VsEP (vestibular evoked potentials) technique.
2. Combine VsEP response data across strains with GWAS (Genome-Wide Association Study) to identify candidate genes involved in the regulation of the vestibular system.

Methods:
Mice (6 wk old) were anesthetized with ketamine 100 mg/kg and xylazine 10 mg/kg and positioned supine with the head mount coupling the cranium securely to a mechanical shaker. Stimuli consisted of linear acceleration (17 pulses/sec) applied to the cranium in the naso-occipital axis. The first (P1) and second (P2) positive and negative response peaks were measured as phenotypes. Mice were selected based on a combined set of classic inbred (CI) and recombinant inbred (RI) strains from the Hybrid Mouse Diversity Panel (HMDP).

Results:
A total of 14 CI and 21 RI were evaluated with an average of range 3-4 mice/strain. A wide range of phenotypic responses were observed. The mean threshold (-12.64 ± 5.31 dB re: 1g/ms) and P2-N2 amplitude at ± 6 dB re: 1g/ms (0.742 ± 0.518 mV) both demonstrated statistical significant variation (ANOVA) in mean VsEP thresholds (p < 0.001) and P2-N2 amplitude (p < 0.001) among the 35 strains. Analysis of the phenotype data reveals a significant peak SNP on Chr18 (p-value = 4.58E−6) corresponding to the DCC gene which has implications for axonal development.

Conclusion:
These data demonstrate significant variation in VsEP response parameters across mouse strains, strongly suggesting the hypothesis that there exists functional variation of vestibular function among strains of mice and the genetic determinants of such variation can be mapped using GWAS.

INTRODUCTION

A growing body of evidence has emerged supporting the contribution of genes to vestibular dysfunction in mammals, including humans and mice (1). A candidate gene approach in humans, either by case-control studies or by the sequencing of selected genes, has been used to search for associated genetic markers (2). However, each of these efforts suffers from the limitations of many human genetic studies, namely insufficient statistical power, difficulty in reproducibility, and difficulty in controlling for environmental factors (3). We propose an alternative strategy to circumvent the aforementioned issues in human studies by utilizing recent innovations in mouse GWAS to comprehensively define the genetic basis underlying vestibular dysfunction in mice with the ultimate goal of candidate gene discovery for study in humans.

The first step in our approach is the evaluation of the gravity receptor function using VsEP (vestibular evoked potential) recordings in different strains of mice and identifying functional variation in VsEP response parameters across strains.

METHODS AND MATERIALS

Mice (6 wk old) were anesthetized with ketamine 100 mg/kg and xylazine 10 mg/kg and positioned supine with the head mount coupled to the cranium and secured to a mechanical shaker (Figure 1). Stimuli consisted of linear acceleration (17 pulses/sec) applied to the cranium in the naso-occipital axis (4). The first (P1) and second (P2) positive and negative response peaks were measured as phenotypes (Figure 2). Mice were selected based on a combined set of classic inbred (CI) and recombinant inbred (RI) strains from the Hybrid Mouse Diversity Panel (HMDP) (3).

RESULTS

A total of 14 CI and 21 RI were evaluated with an average of 3-4 mice/strain. A wide range of phenotypic responses were observed. The mean threshold (-12.64 ± 5.31 dB re: 1g/ms) and P2-N2 amplitude at ± 6 dB re: 1g/ms (0.742 ± 0.518 mV) both demonstrated statistical significant variation (ANOVA) in mean VsEP thresholds (p < 0.001) and P2-N2 amplitude (p < 0.001) among the 35 strains (Figure 3).

EMMA (Efficient Mixed Model Association) analysis of the phenotype data from these 35 strains reveals a significant peak SNP on Chr18 (p-value = 4.58E−6) as shown on the Manhattan Plot (Figure 4) and Regional Plot (Figure 5).

DISCUSSION

VsEPs provide quantifiable, sensitive, and reproducible information about the mouse vestibular system with proven evidence of strain variation that serves well in our association mapping strategy. Our preliminary data successfully demonstrates the novel application of mouse GWAS in comprehensively defining the genetic variation associated with vestibular function in mammals.

By about one third of the HMDP was possible to map the first candidate gene, demonstrating how powerful is this methodology not only for hearing loss, but also to comprehensively define candidates genes for vestibular disorders in mice.

CONCLUSIONS

These data demonstrate significant variation in VsEP response parameters across mouse strains, strongly suggesting the hypothesis that there exists functional variation of vestibular function among strains of mice and the genetic determinants of such variation can be mapped using GWAS.

REFERENCES