Determining Phrike (Phrke) Phenotype Causing Mutation through Micro-array Assay Analysis

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Abstract

Spontaneous mutation in mice have been a driving force in the generation of model of human disease and in assigning genes to complex physiological processes. A prominent example of this is our understanding of the myelination of neurons. Mice with changes in myelination are easily detected because of distinctive motor/behavioral changes and comprise the largest subgroup of neurological mutant lines. We identified a mouse in our colony at UNC with a phenotype common to many mice which have been shown to have defective myelination, namely a "shivering" phenotype early in life. We have assigned this mutant mouse line the name "Phrike." The mice which developed this phenotype are inbred and the strain is designated 129S6 (129). As a first step to identification of the gene carrying the mutation, we backcrossed it onto a C57BL/6N (B6) genetic background. With each generation, the mutant mice are expected to carry less 129 DNA, and more B6 DNA. Furthermore, it is expected that DNA in linkage with Phrike will be of 129 origin. We now report the use of a micro-array platform designed to distinguish between the genome of inbred mouse lines, MiniMUGA, to localize the causative mutation in our Phrike mice. Specifically, we show that the Phrike phenotype maps to chromosome 17, and two possible regions are identified, which harbor genes known to play critical roles in myelination. We discuss the possibility that Phrike represents a novel allele of one of these genes or a new chromosome 17 gene involved in neuronal function.

Introduction

A Microarray platform was used to match DNA of Phrike mice to probes of known markers to test the presence of specific genetic constructs. The microarray platform that was used was the MiniMUGA[2], a genotyping array that provides SNP markers on an illumine Infinium platform[3]. This genotyping array provides a highly cost-efficient tool for detecting heterozygous regions and discriminating between haplotypes in homozygous regions. Additionally, it provides broad discrimination between most inbred strains, supporting mapping of dozens of different reduced complexity crosses (RCCs) involving multiple substrains, and detects presence of many genetic constructs.

This is done by conjugating invariable oligonucleotide probes (of 50bp length) to silica beads, which are addressed to wells on the illumine Infinium HD platform[3]. Sample DNA are fluorescently labeled with nucleotides, and are hybridized to the oligonucleotide probes. Based on the signal intensity of the fluorophores, the genotype (AA, AB, BB) is determined (**Figure 1**).

Results

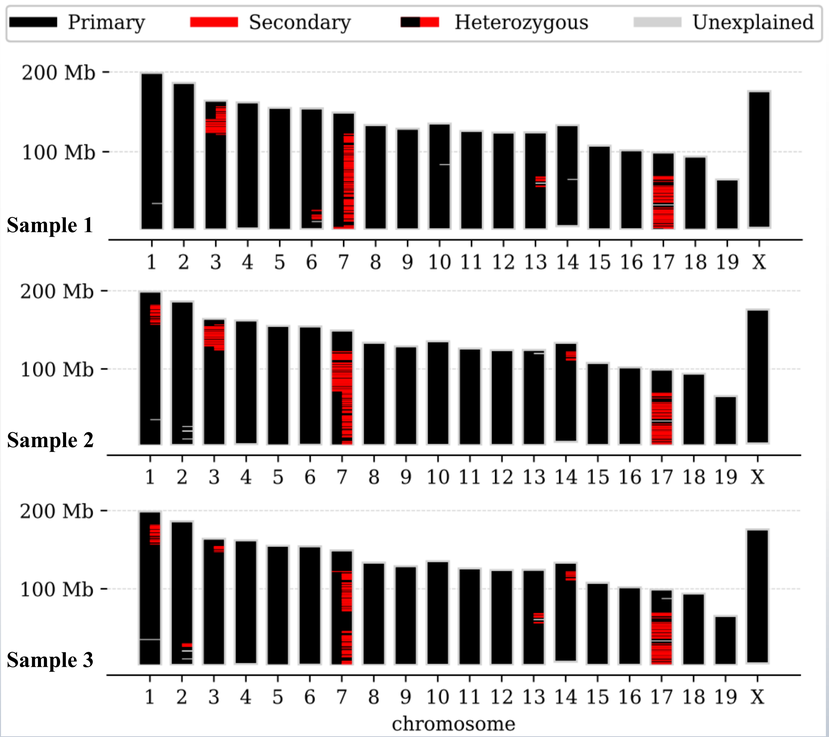

Figure 2: miniMUGA results

As the gene exhibits a recessive phenotypic pattern, this means that the gene must be homozygous 129 (marked fully red). As the only chromosome that is homozygous 129 for all three sample studied is chromosome 17, we deduced that the gene of interest lies on chromosome 17.

gene	alias	Inheritance	Location	
			sequence map	genetic map
Qk(qk-shk)	quaking; quaking shaking	recessive	Chr17:10206471-10319361 bp	Chr17, 7.75 cM
Qk(qk-2J)	quaking 2 jackson	recessive	Chr17:10206471-10319361 bp	Chr17, 7.75 cM
Mog(tm1Dpd)	myelin oligodendrocyte glycoprotein	Autosomal Dominant	Chr17:37010743-37023398 bp	Chr17, 19.16 cM, cytoband C
H2 (dlAb1-Ea)	Histocompatibility-2, Targeted deletion, H2 complex	N/A	unknown	Chr17, cytoband B-C

Table 1: Known Chromosome 17 genes essential for myelination and/or motor neuron function in mice

We used MGI[4] and other data bases that list genes in which mutations have been demonstrated to result in phenotypes similar to that of Phrike, to determine whether any of these gene resided in the regions of chromosome 17 that remain homozygous for 129 DNA. Several such genes were identified and are shown in **Table 1**. The Mog locus is inherited as an autosomal dominate trait. This makes it a less likely candidate. However, it is possible that Phrike represents a less severe mutant allele of Mog and thus the phenotype becomes apparent only in homozygous animals.
The more likely candidate is Qk. MGI lists numerous allele of the Qk gene.

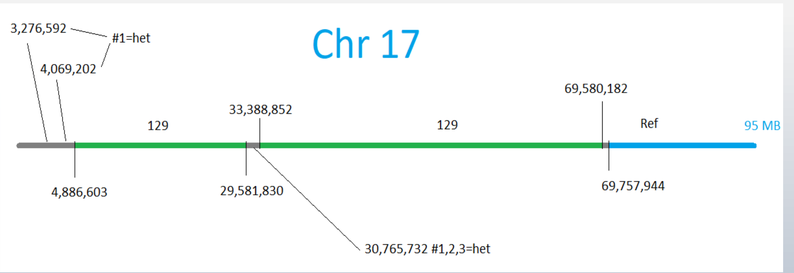

Figure 3: Chromosome 17 sequence breakdown

The reference sequence (C57BL/6N) are marked in blue, the 129 sequence in green, and the heterozygous segments in grey. Majority of chromosome 17 was 129, which makes it difficult to figure out which part of the sequence had the Phrike mutation. However, using commonly known mutation sites for Chromosome 17 (**Table 1),** could narrow down mutation sites.

Methodology

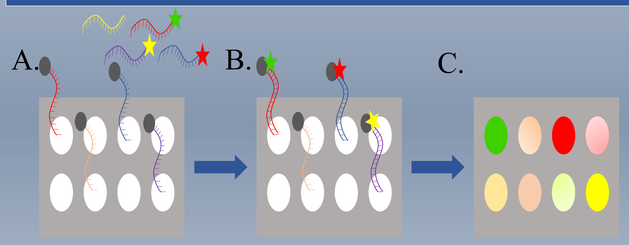

Figure 1a: Infinium Assay used in miniMUGA for 129

A. Oligonucleotide probes (colors represent different probes) for specific genetic constructs are conjugated to silica beads (grey). Mutated sample DNA (of 129 origin) have fluorescently labeled nucleotides (Stars) and DNA of C57BL/6N are not labeled fluorescently.
B. A fluorescence signal can only be found when the 3' end of the fluorescently marked primer pair is perfectly matched with the "mismatched" primer pair (probe).
C. The relative bsignal intensity from the fluorophores at target nucleotide is processed into AA, AB, or BB using Illumina BeadStudio software. (Ex: AA= bright green, AB= right yellow, BB= bright red)

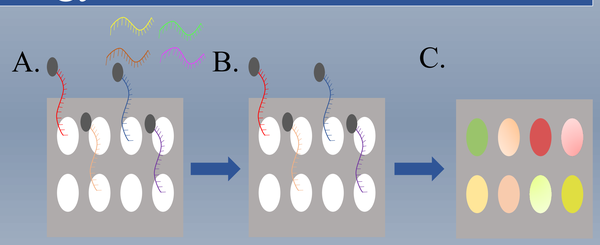

Figure 1b: Infinium Assay used in miniMUGA for C57BL/6N (B6) mouse

A. Oligonucleotide probes (colors represent different probes) for specific genetic constructs are conjugated to silica beads (grey). Mutated sample DNA (of 129 origin) have fluorescently labeled nucleotides (Stars) and DNA of C57BL/6N are not labeled fluorescently.
B. No fluorescence signal is found as the DNA did not match the probes.
C. The relative signal intensity from the fluorophores at target nucleotide is processed into AA, AB, or BB using Illumina BeadStudio software. (Ex: AA= bright green, AB= right yellow, BB= bright red)

Conclusion/ Future Direction

- The Phrike gene lies within Chromosome 17 on a segment that is of 129 origin.
- The inheritance pattern of the mutation is recessive.

- Further Research will be done to:

1. Verify the MUGA results placing Phrike in one of the two regions on Chromosome 17 using using strain specific PCR primers sets.
2. Reduce the interval of 129 in which Phrike resides, i.e. localize to one of the two DNA segments (would require more backcrossing with C57BL/6N mice).
3. Eliminate Mog (tm1Dpd) by sequencing Mog cDNA/gene from the mutants (as it has a different inheritance pattern).
4. Eliminate Qk by sequence/expression analysis in relevant tissues.
5. If Phrike mice have wild type Mog and Qk, identify Phrike by sequence the homozygous mutant Phrike mouse and a wildtype 129 mouse.
6. If Phrike is an allele of Qk or Mog, identify the mutation and compare the phenotype to that of other mouse lines carrying other mutations in these genes.

References

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