# Genetic analysis of RNA editing in the Diversity Outbred mice


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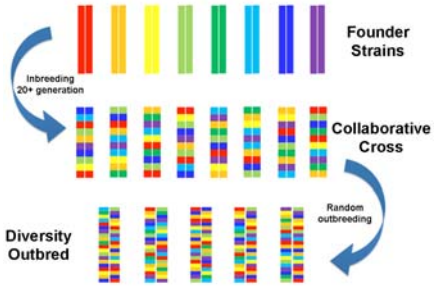
## Abstract


**RNA editing refers to a co-/post- transcriptional process that alters the sequence of RNA. There are two types of editing known in mammals: A->I and C->U editing. A->I editing is mediated by the ADAR family of enzymes and occurs most frequently in neuronal tissues. C->U editing occurs in the liver and small intestine where editing of the transcript encoding APOB is mediated by the editing enzyme APOBEC1.**

**Recently, hundreds of new RNA editing targets have been reported. However, the mechanisms that regulate and determine specificity of editing are not well understood. We used a novel high-resolution mapping population of mice, the Diversity Outbred (DO), to identify editing sites that are subject to genetic variation and mapped polymorphic loci that alter the editing ratio at specific C->U and A->I editing sites.**

**Our results suggest that editing ratio is a precisely regulated quantitative trait and that polymorphisms responsible for RNA editing that can be directly identified in DO mice.**


## The development strategy of the Diversity Outbred mice




## Pipeline for the genetic analysis of RNA editing

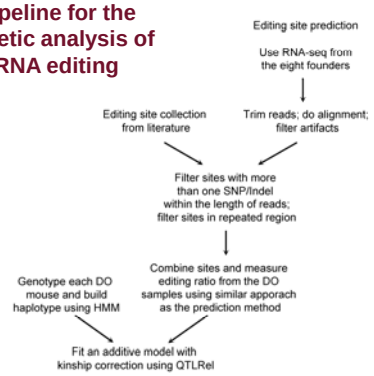



**Fig. 1, QTL mapping for editing site at *Apob*.**

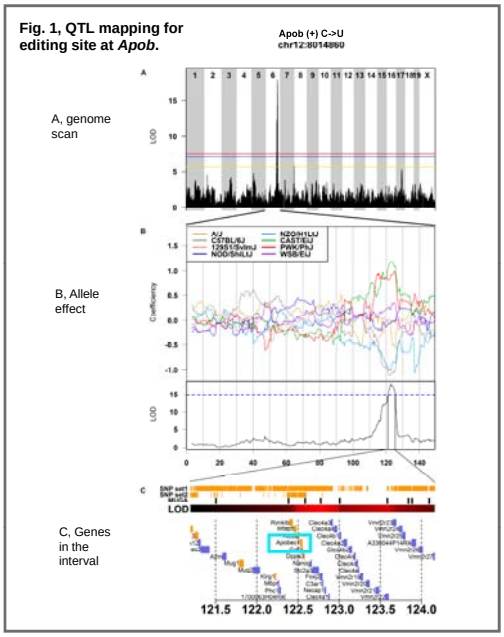
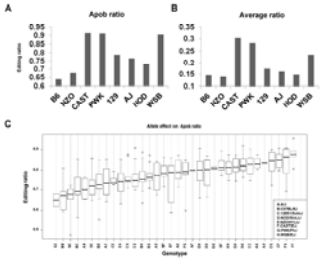



**Fig. 2, Editing ratio distribution at different genotypes.**

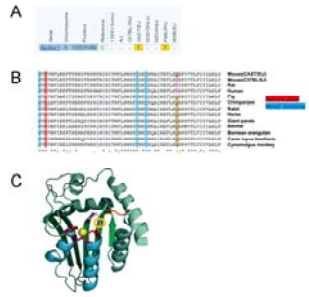



A, Distribution of the site at *Apob* in the eight founders.

B, Distribution of 49 other C->U sites in the eight founders.

C, Distribution of the *Apob* site across 36 states in the DO samples.

**Fig. 3, A single amino acid change (R to Q) shared by CAST and PWK increases the catalytic activity of APOBEC1.**




A, The location of the amino acid.
B, Sequence comparison in animals.
C, Position of the amino acid in the three dimensional structure.

**Fig. 4, A large insertion in B6 and NZO induces aberrant splicing of *Apobec1*, which reduces the editing efficiency in B6 and NZO. A SNP increases the expression of the long reference isoform of *Apobec1*.**

A, The location of the insertion and the SNP (B).
C, Distribution of the coverage at the insertion and the SNP in the eight founders.

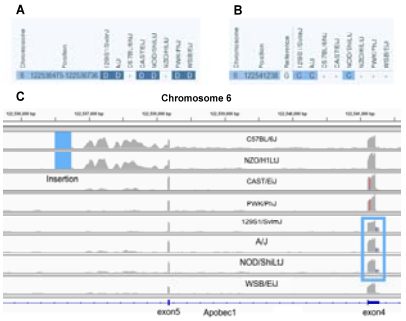



**Fig. 5, The aberrant isoform of *Apobec1* represents >50% of the total isoforms. The long isoform of *Apobec1* expresses highly in 129, AJ and NOD.**

A, A model for the different isoforms of *Apobec1*.
B, Quantification of the isoforms of *Apobec1*.

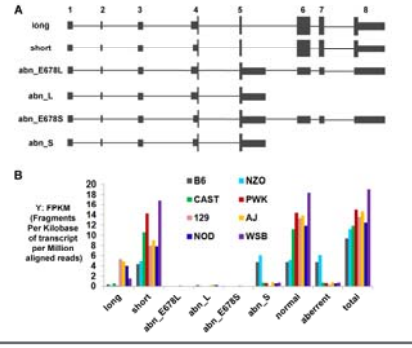



**Fig. 6, QTL mapping for the site at *Cds2*.**

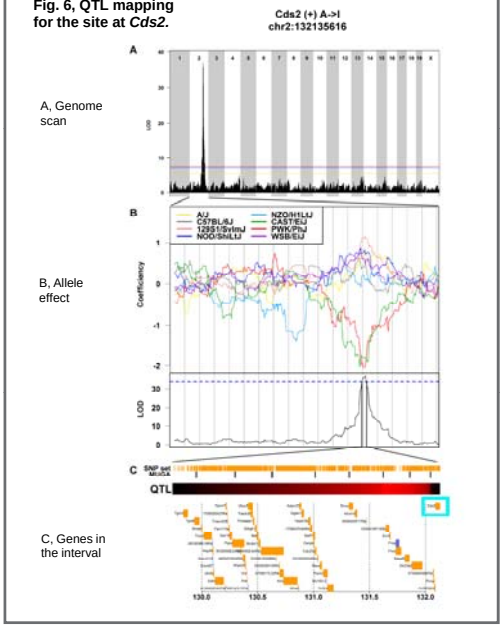
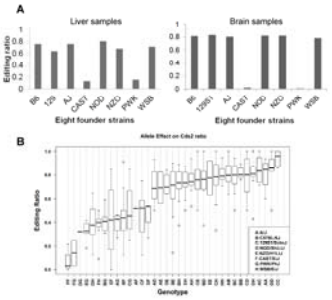


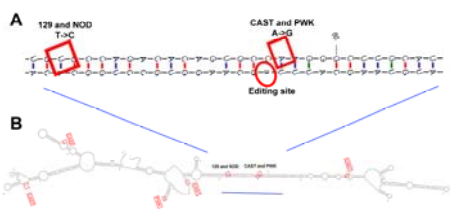

**Fig. 7, Editing ratio distribution for site at *Cds2* at different genotypes.**




A, Distribution in the liver and the brain of the eight founder strains.

B, Distribution of editing ratio across 36 states in DO samples.

**Fig. 8, The SNP shared by CAST and PWK is next to the editing site at *Cds2*.** A, Enlarged region around the editing site. B, Global structure.




## Conclusions

**We discovered consistent and strong genetic effects for 50 C->U editing sites.**

1. **One trans-QTL at chromosome 6 including APOBEC1 was discovered.**
2. **An amino acid substitution in APOBEC1 increases its catalytic efficiency.**
3. **A large intronic insertion produces a non-functional protein and reduces its function.**
4. **A SNP increases the expression of the long isoform of *Apobec1*.**

**Several local genetic effects were found for A->I editing.**

1. **Six cis-QTLs were discovered for six A->I editing sites.**
2. **The polymorphisms far from editing site in the linear sequence are in the neighborhood of the editing site in the secondary structure and alter the editing efficiency.**