

***Chrna4* Cas9-KO Strategy**

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Project Overview

Project Name

Chrna4

Project type

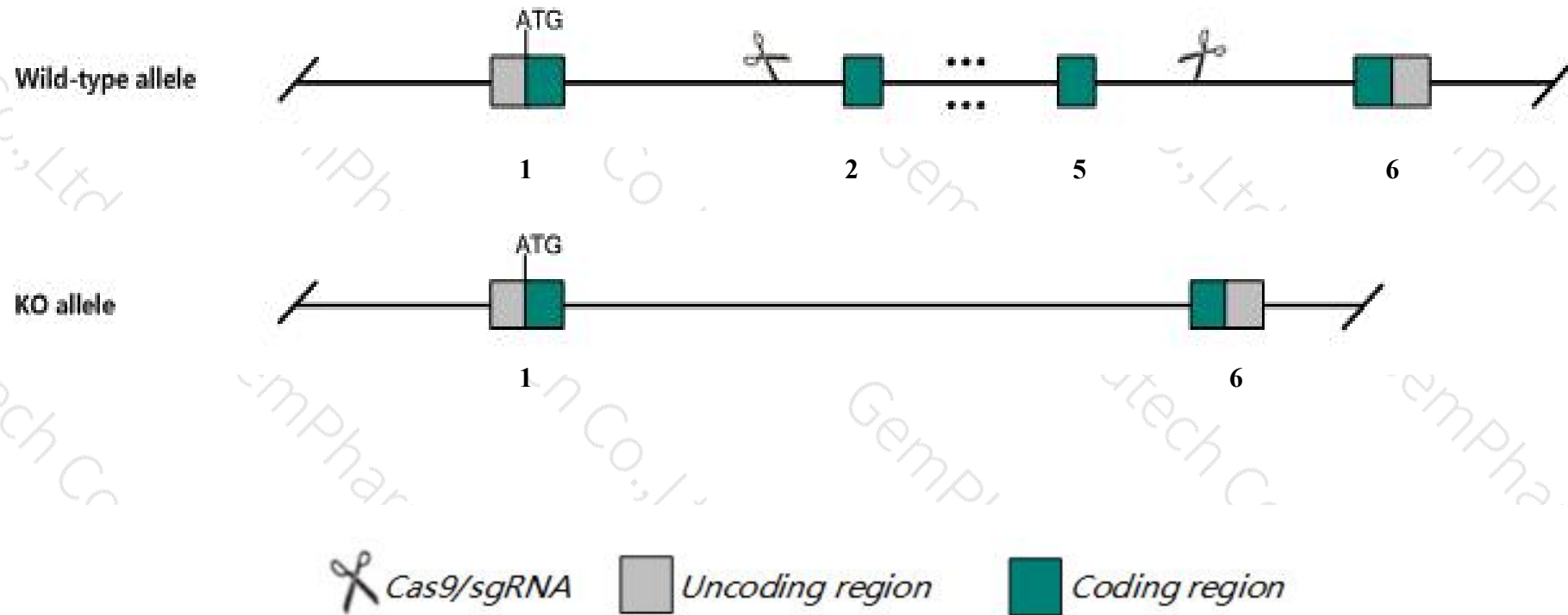
Cas9-KO

Strain background

C57BL/6J

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Chrna4* gene. The schematic diagram is as follows:



- The *Chrna4* gene has 5 transcripts. According to the structure of *Chrna4* gene, exon2-exon5 of *Chrna4-201* (ENSMUST00000067120.13) transcript is recommended as the knockout region. The region contains 1682bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Chrna4* gene. The brief process is as follows: sgRNA was transcribed in vitro. Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6J mice.

- According to the existing MGI data, Nullizygous mice may show reduced chemically-elicited analgesia, susceptibility to seizures, increased anxiety, and altered behavioral responses to nicotine or a new environment. Homozygotes for any of several knock-in alleles exhibit altered nervous system physiology and/or sensitivity to nicotine.
- The *Chrna4* gene is located on the Chr2. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

Chrna4 cholinergic receptor, nicotinic, alpha polypeptide 4 [Mus musculus (house mouse)]

Gene ID: 11438, updated on 31-Jan-2019

Summary



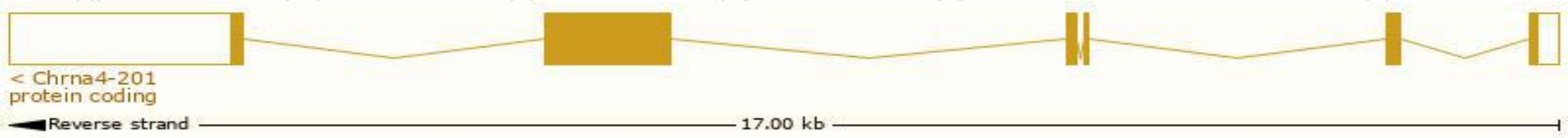
Official Symbol	Chrna4 provided by MGI
Official Full Name	cholinergic receptor, nicotinic, alpha polypeptide 4 provided by MGI
Primary source	MGI:MGI:87888
See related	Ensembl:ENSMUSG00000027577
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Acra-4, Acra4, EBN1, ENFL1
Expression	Biased expression in kidney adult (RPKM 15.0), whole brain E14.5 (RPKM 7.5) and 10 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

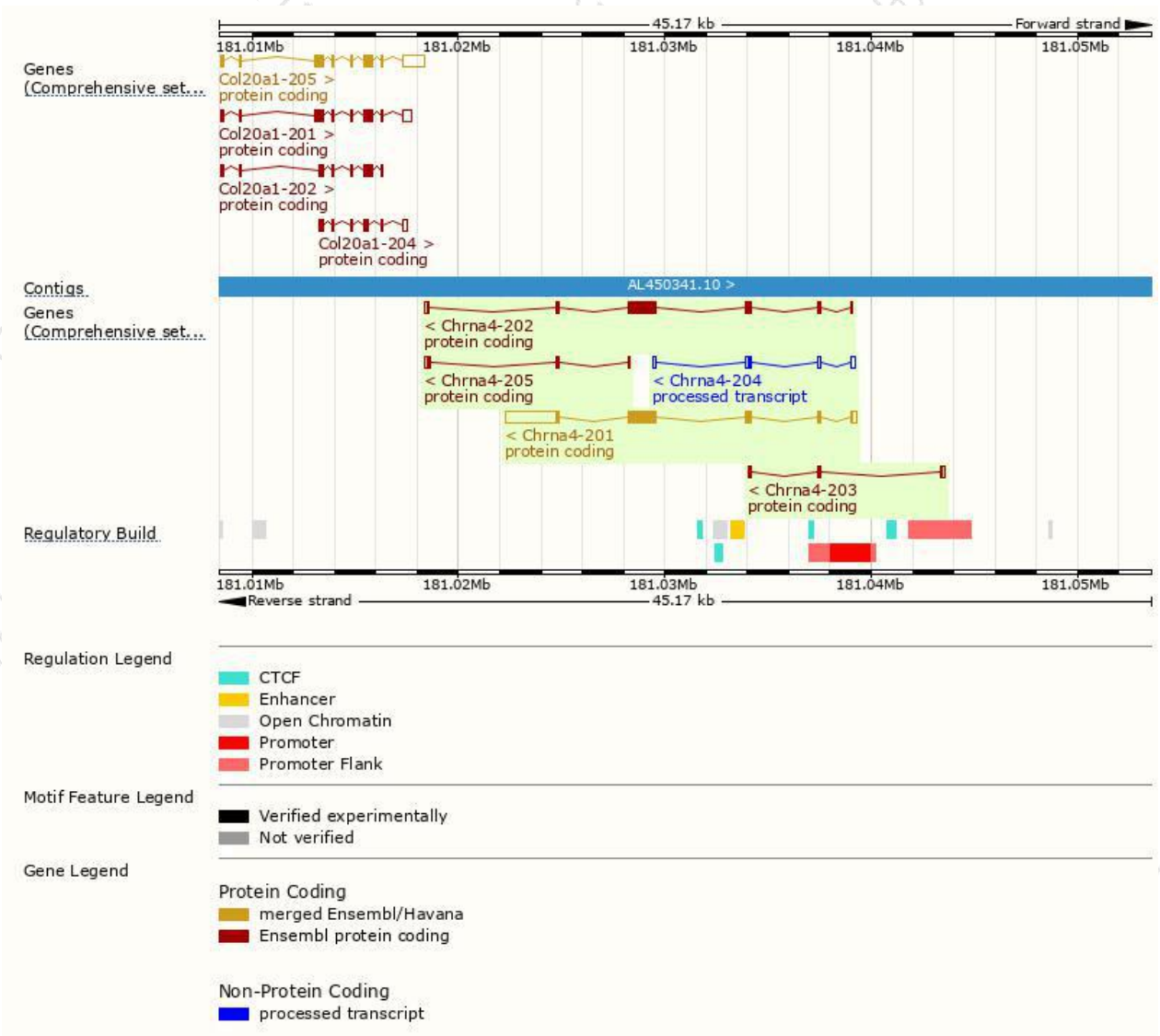
The gene has 5 transcript,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Chrna4-201	ENSMUST00000067120.13	4565	629aa	Protein coding	CCDS17192	O70174 Q53YK0	TSL:1 GENCODE basic APPRIS P2
Chrna4-202	ENSMUST00000108851.7	2071	641aa	Protein coding	-	B7ZBU7	TSL:5 GENCODE basic APPRIS ALT2
Chrna4-205	ENSMUST00000198922.1	519	125aa	Protein coding	-	A0A0G2JFP3	CDS 5' incomplete TSL:5
Chrna4-203	ENSMUST00000124400.1	363	78aa	Protein coding	-	B7ZBV1	CDS 3' incomplete TSL:5
Chrna4-204	ENSMUST00000135766.4	712	No protein	Processed transcript	-	-	TSL:3

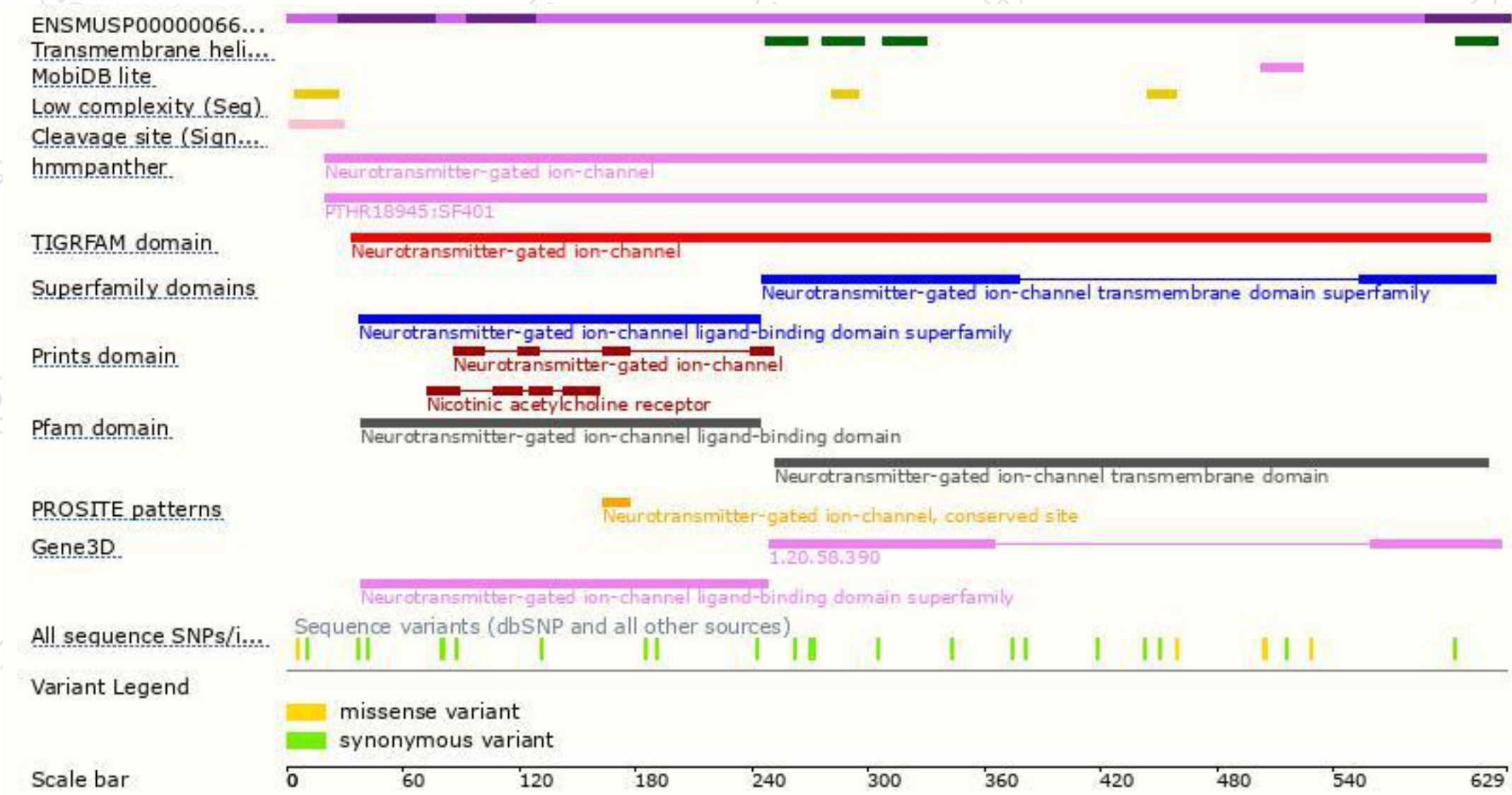
The strategy is based on the design of *Chrna4-201* transcript,The transcription is shown below



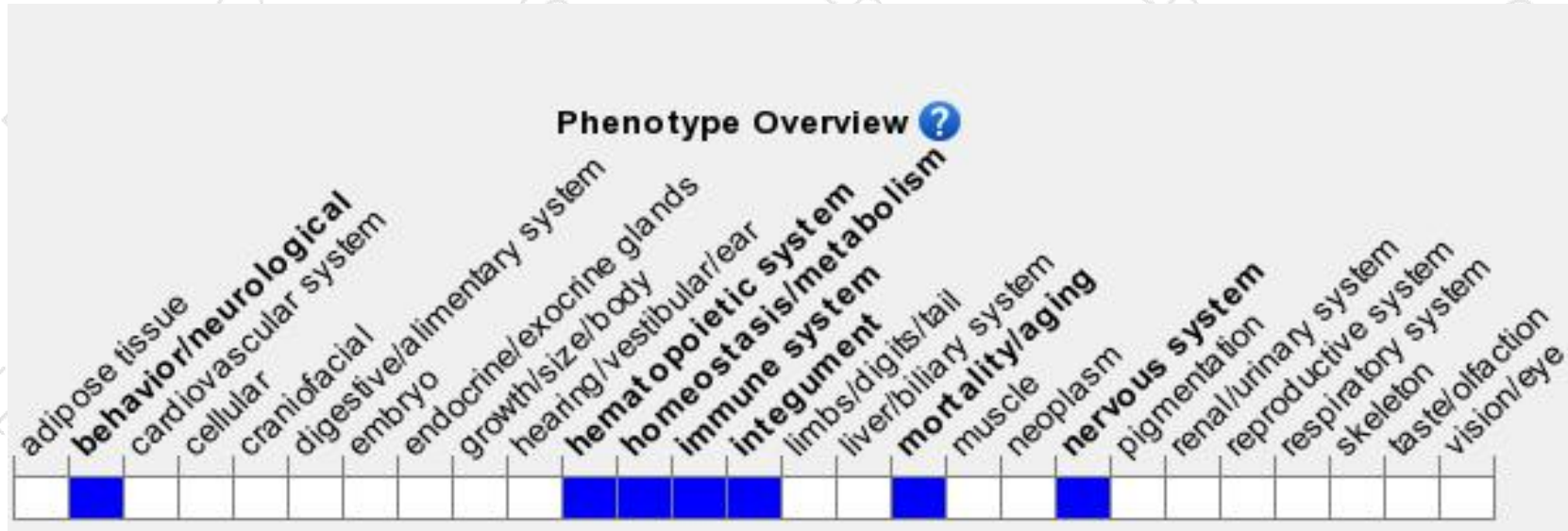
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).

According to the existing MGI data, Nullizygous mice may show reduced chemically-elicited analgesia, susceptibility to seizures, increased anxiety, and altered behavioral responses to nicotine or a new environment. Homozygotes for any of several knock-in alleles exhibit altered nervous system physiology and/or sensitivity to nicotine.

If you have any questions, you are welcome to inquire.

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