

# *Nf1* Cas9-CKO Strategy

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# Project Overview

**Project Name**

*Nf1*

**Project type**

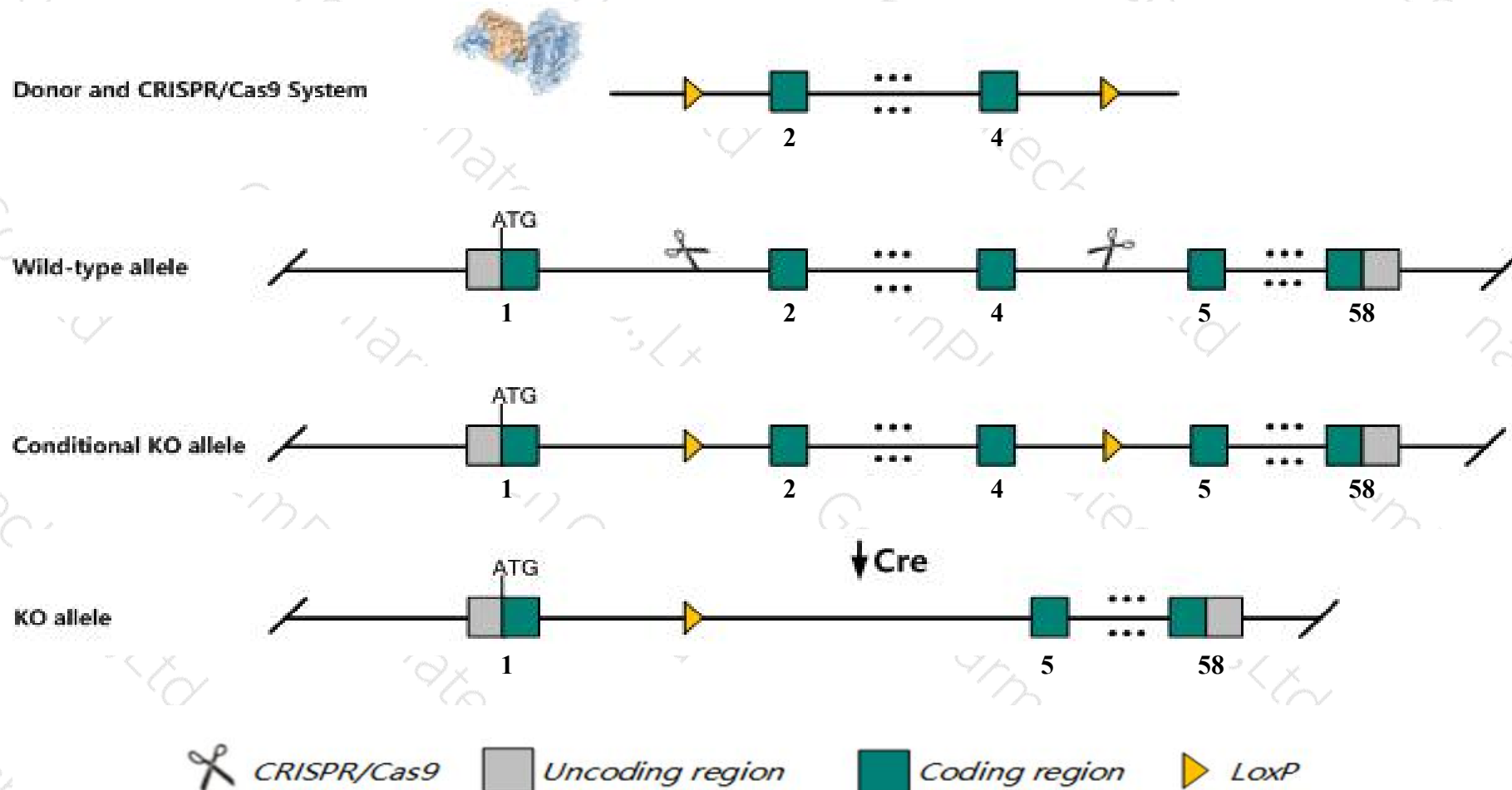
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nf1* gene. The schematic diagram is as follows:



# Technical routes

- The *Nf1* gene has 9 transcripts. According to the structure of *Nf1* gene, exon2-exon4 of *Nf1-201* (ENSMUST00000071325.8) transcript is recommended as the knockout region. The region contains 419bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Nf1* gene. The brief process is as follows: CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt 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/6JGpt mice.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

- According to the existing MGI data, Homozygous embryos die by day 14.5 with enlarged head and chest, pale liver, microphthalmia, cardiac defects and delayed organ development. Heterozygotes have elevated astrocyte number, predisposition to multiple tumor types and learning/memory deficits.
- The *Nf1* gene is located on the Chr11. 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 biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.



# Gene information (NCBI)

## Nf1 neurofibromin 1 [Mus musculus (house mouse)]

Gene ID: 18015, updated on 9-Apr-2019

### Summary



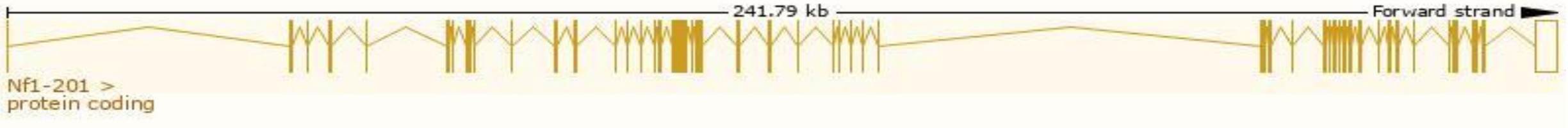
<b>Official Symbol</b>	Nf1 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	neurofibromin 1 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:97306</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000020716</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	AW494271, Dsk9, E030030H24Rik, Mhdadsk9, Nf-1
<b>Expression</b>	Ubiquitous expression in cerebellum adult (RPKM 6.5), whole brain E14.5 (RPKM 6.4) and 28 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

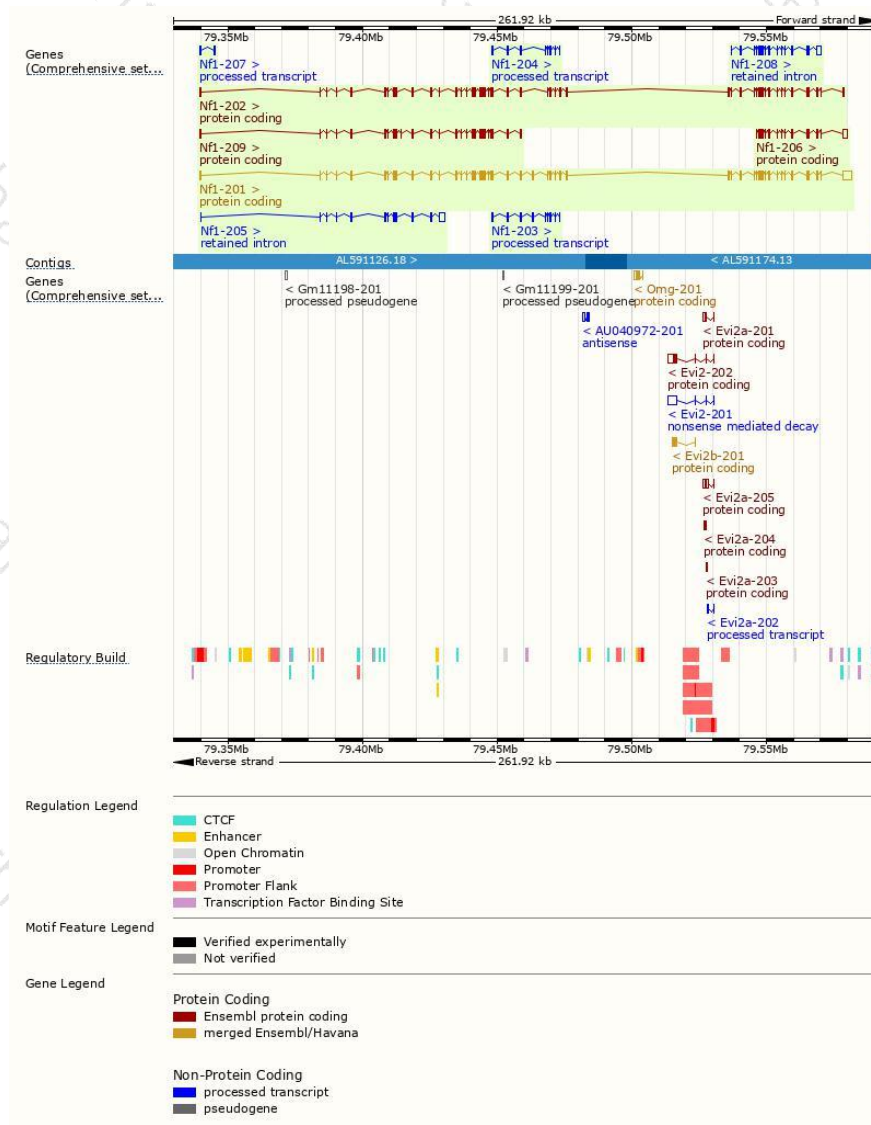
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nf1-201	<a href="#">ENSMUST00000071325.8</a>	11917	<a href="#">2841aa</a>	Protein coding	<a href="#">CCDS25119</a>	<a href="#">Q04690</a>	TSL:2 GENCODE basic APPRIS P2
Nf1-202	<a href="#">ENSMUST00000108251.8</a>	9217	<a href="#">2820aa</a>	Protein coding	-	<a href="#">Q04690</a>	TSL:5 GENCODE basic APPRIS ALT2
Nf1-209	<a href="#">ENSMUST00000219057.1</a>	4354	<a href="#">1382aa</a>	Protein coding	-	<a href="#">A0A1W2P863</a>	CDS 3' incomplete TSL:5
Nf1-206	<a href="#">ENSMUST00000137997.1</a>	4133	<a href="#">826aa</a>	Protein coding	-	<a href="#">Q5SYH9</a>	CDS 5' incomplete TSL:1
Nf1-203	<a href="#">ENSMUST00000122917.7</a>	1178	No protein	Processed transcript	-	-	TSL:1
Nf1-204	<a href="#">ENSMUST00000130979.1</a>	1115	No protein	Processed transcript	-	-	TSL:1
Nf1-207	<a href="#">ENSMUST00000145839.1</a>	721	No protein	Processed transcript	-	-	TSL:2
Nf1-208	<a href="#">ENSMUST00000146699.1</a>	4053	No protein	Retained intron	-	-	TSL:5
Nf1-205	<a href="#">ENSMUST00000131800.1</a>	3851	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Nf1-201* transcript,The transcription is shown below

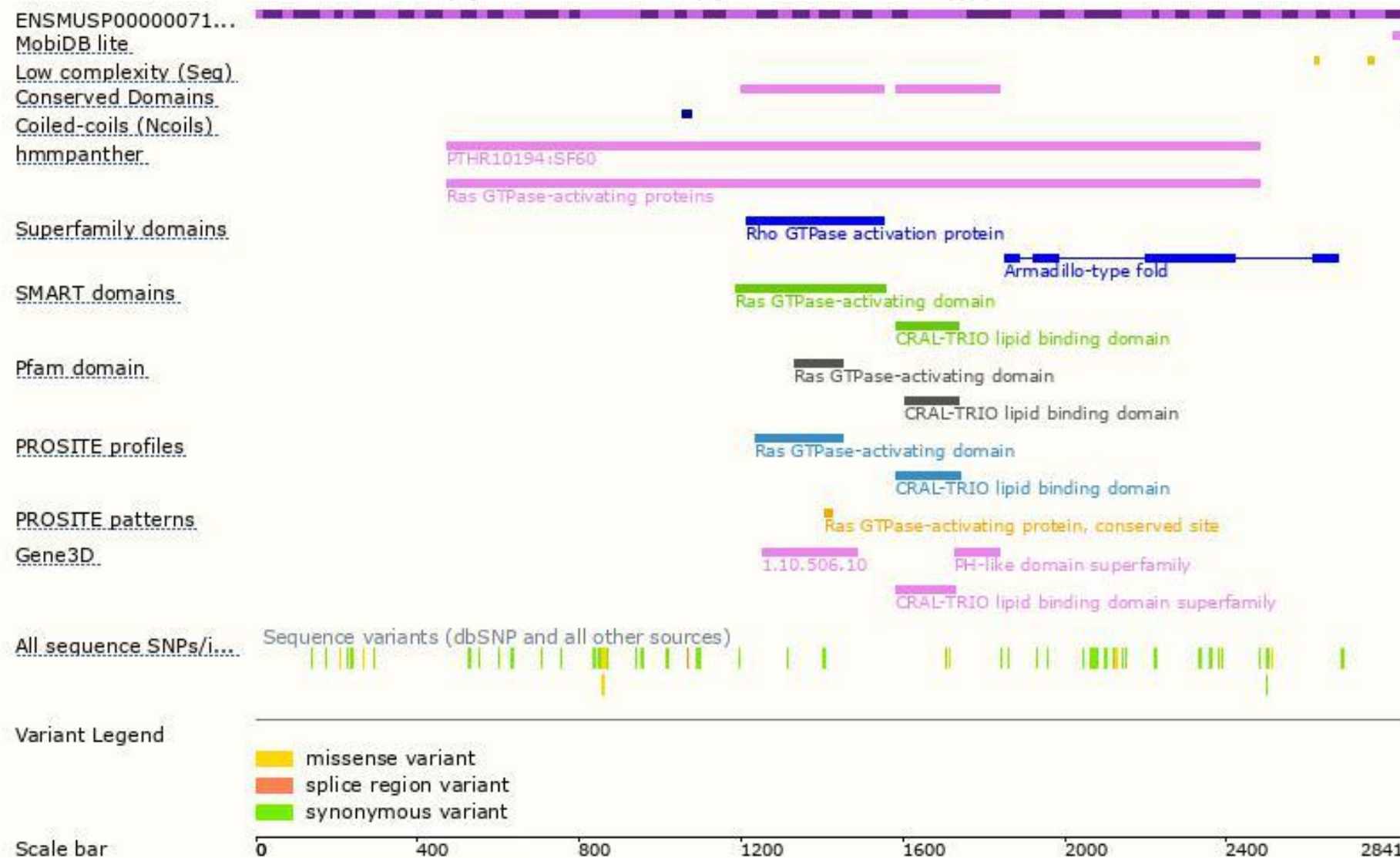


# Genomic location distribution

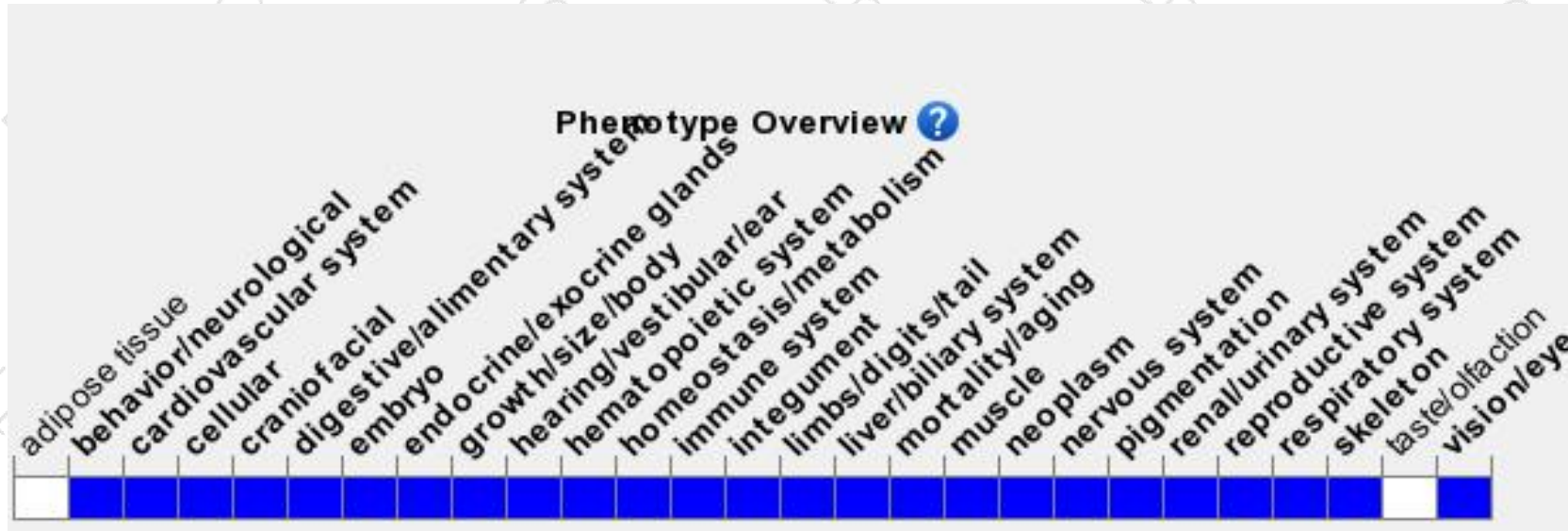




# Protein domain



# 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, Homozygous embryos die by day 14.5 with enlarged head and chest, pale liver, microphthalmia, cardiac defects and delayed organ development. Heterozygotes have elevated astrocyte number, predisposition to multiple tumor types and learning/memory deficits.

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

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