Name of Mouse model or mutation:
F13A1-L35V-EM1-B6

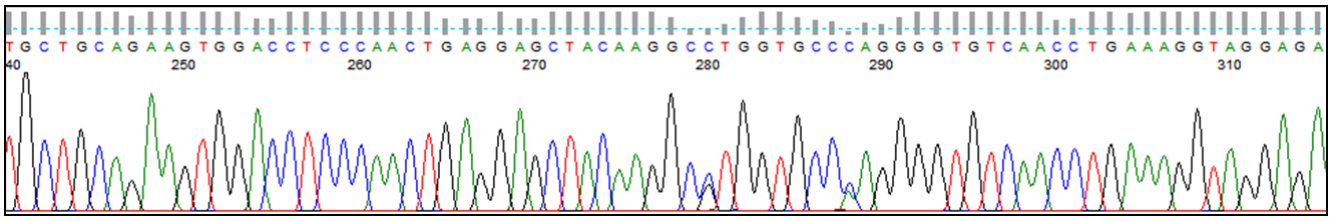
Description:
Point mutation model made using CRISPR/Cas9.

Type of mutation:
SNP: L35V

Sequence details
F13a1 WT
CAGGGGACACACTCCTTCACTGGGGTAATCCTCACTGCCACCTCCCAGTTATCATGTTTTGTGTGTGG
CTTACAATGTATTGTCTGGGAGCTGCCATGTACGGTTTGCATCTCTGCCTCTATTTTAGAATCCTAGCC
ACAAGTGGTACCCATTTCACATTCCCGCCCCATGAACTCTCTTCTCCCTTCCCAAGGATCCAGTAAAG
CTGAGAATGTCAGATACTCCAGCAAGCACCTTTGGGGGGAGGCGAGCAGTCCCGCCCAATAACTCC
AATGCTGCAGAAGTGGACCTCCCAACTGAGGAGCTACAAGGCCTGGTGCCAAGGGGTGTCAACCTG
AAAGGTAGGAGAAGCATACCACTGTGTCTACTAGAGCCGGACCTCATTTCCCATGAGGGATCTGTGT
CAGGACCCTCCTCCACAAAGATTTACAAAAATATGTCCCCTTGTAGTCTCTGGTGCCCACCTAAGAAA
GTCTCCAAAACCCAAGCTGTAGAAATACCAGAGAAGCTGAAAGGACAGTCCTAGTTTGGGCCTT

F13a1 L35V
CAGGGGACACACTCCTTCACTGGGGTAATCCTCACTGCCACCTCCCAGTTATCATGTTTTGTGTGTGG
CTTACAATGTATTGTCTGGGAGCTGCCATGTACGGTTTGCATCTCTGCCTCTATTTTAGAATCCTAGCC
ACAAGTGGTACCCATTTCACATTCCCGCCCCATGAACTCTCTTCTCCCTTCCCAAGGATCCAGTAAAG
CTGAGAATGTCAGATACTCCAGCAAGCACCTTTGGGGGGAGGCGAGCAGTCCCGCCCAATAACTCC
AATGCTGCAGAAGTGGACCTCCCAACTGAGGAGCTACAAGGC**G**TGGTGCC**C**AGGGGTGTCAACCTG
AAAGGTAGGAGAAGCATACCACTGTGTCTACTAGAGCCGGACCTCATTTCCCATGAGGGATCTGTGT
CAGGACCCTCCTCCACAAAGATTTACAAAAATATGTCCCCTTGTAGTCTCTGGTGCCCACCTAAGAAA
GTCTCCAAAACCCAAGCTGTAGAAATACCAGAGAAGCTGAAAGGACAGTCCTAGTTTGGGCCTT

F13a1 L35V Heterozygous F1 animal sequence trace:



Nucleotide Alignment:

```
                    *        20         *        40         *        60         *        80         *       100         *
F13a1_WT    : GATCCAGTAAAGCTGAGAATGTCAGATACTCCAGCAAGCACCTTTGGGGGGAGGCGAGCAGTCCCGCCCAATAACTCCAATGCTGCAGAAGTGGACCTCCCAACTGAGGAGCTA : 114
F13a1_L35V  : GATCCAGTAAAGCTGAGAATGTCAGATACTCCAGCAAGCACCTTTGGGGGGAGGCGAGCAGTCCCGCCCAATAACTCCAATGCTGCAGAAGTGGACCTCCCAACTGAGGAGCTA : 114
              GATCCAGTAAAGCTGAGAATGTCAGATACTCCAGCAAGCACCTTTGGGGGGAGGCGAGCAGTCCCGCCCAATAACTCCAATGCTGCAGAAGTGGACCTCCCAACTGAGGAGCTA

                 120         *       140         *       160         *       180         *       200         *       220
F13a1_WT    : CAAGGCCTGGTGCCAAGGGGTGTCAACCTGAAAGGTAGGAGAAGCATACCACTGTGTCTACTAGAGCCGGACCTCATTTCCCATGAGGGATCTGTGTCAGGACCCTCCTCCACA : 228
F13a1_L35V  : CAAGGCGTGGTGCCCAGGGGTGTCAACCTGAAAGGTAGGAGAAGCATACCACTGTGTCTACTAGAGCCGGACCTCATTTCCCATGAGGGATCTGTGTCAGGACCCTCCTCCACA : 228
              CAAGGC TGGTGCC AGGGGTGTCAACCTGAAAGGTAGGAGAAGCATACCACTGTGTCTACTAGAGCCGGACCTCATTTCCCATGAGGGATCTGTGTCAGGACCCTCCTCCACA

               *       240         *       260         *       280         *       300         *       320         *       340
F13a1_WT    : AAGATTTACAAAAATATGTCCCCTTGTAGTCTCTGGTGCCCACCTAAGAAAGTCTCCAAAACCCAAGCTGTAGAAATACCAGAGAAGCTGAAAGGACAGTCCTAGTTTGGGCCT : 342
F13a1_L35V  : AAGATTTACAAAAATATGTCCCCTTGTAGTCTCTGGTGCCCACCTAAGAAAGTCTCCAAAACCCAAGCTGTAGAAATACCAGAGAAGCTGAAAGGACAGTCCTAGTTTGGGCCT : 342
              AAGATTTACAAAAATATGTCCCCTTGTAGTCTCTGGTGCCCACCTAAGAAAGTCTCCAAAACCCAAGCTGTAGAAATACCAGAGAAGCTGAAAGGACAGTCCTAGTTTGGGCCT
```

Predicted Protein Alignment:

```
                     *        20         *        40         *        60         *        80         *
F13a1_WT    : MSDTFASTFGGRRAVPPNNSNAAEVDLPTEELQGLVPRGVNLKGRRSIPLCLLEPDLISHEGSVSGPSSTKIYKNMSPCSLWCPPKKVSKTQAVEIPE :  98
F13a1_L35V  : MSDTFASTFGGRRAVPPNNSNAAEVDLPTEELQGVVPRGVNLKGRRSIPLCLLEPDLISHEGSVSGPSSTKIYKNMSPCSLWCPPKKVSKTQAVEIPE :  98
              MSDTFASTFGGRRAVPPNNSNAAEVDLPTEELQG VPRGVNLKGRRSIPLCLLEPDLISHEGSVSGPSSTKIYKNMSPCSLWCPPKKVSKTQAVEIPE
```

QC strategy employed:

Genomic DNA was extracted from ear clip biopsies and amplified in a PCR reaction using the following conditions/primer sequences:

Geno_F13a1_F1	CAGGGGACACACTCCTTCAC
Geno_F13a1_R1	AAGGCCCAAACTAGGACTGT
Taq Polymerase used	Roche Expand Long Range dNTPack
Annealing Temperature (°C)	63
Elongation time (min)	1
WT product size (bp)	536
Mutant product size (bp)	536

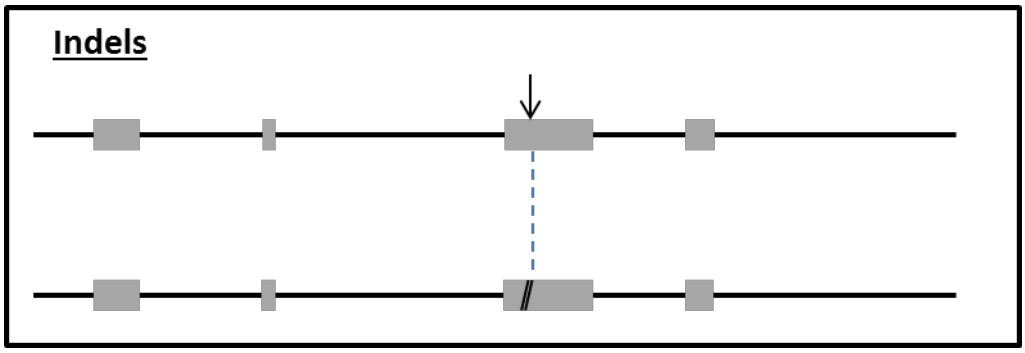
All amplicons were sent for Sanger sequencing to check for integration of the donor oligo sequence at the target site.

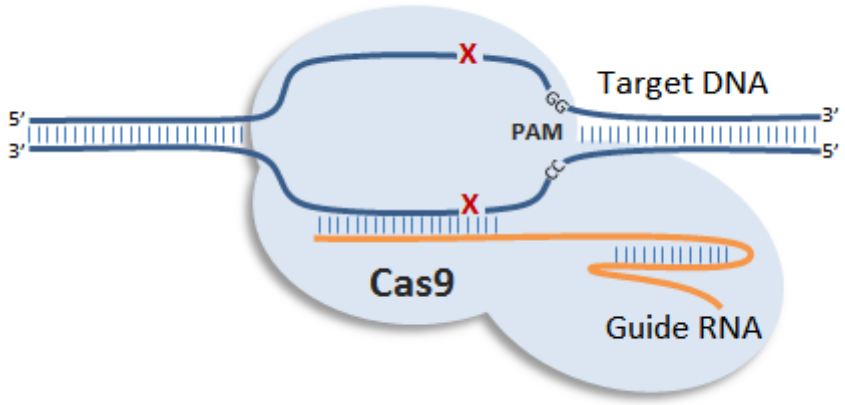
Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligos were inserted once on target into the genome. The following Taqman assay was used to detect the donor sequence:

Assay name	F13A1-L35V-DONOR2-MUT1
Forward Primer	ACACCCCTGGGCACCAC
Reverse Primer	TCCCGCCCAATAACTCCAATG
Probe	TAGCTCCTCAGTTGGGAGGTCCA
Label	FAM-BHQ1

F13A1-L35V Genotyping Strategy

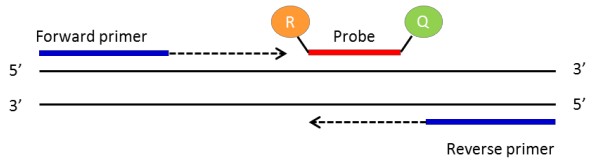
Animals have been engineered using the CRISPR/Cas9 technology. Most of the knockout alleles generated through this method will be obtained by deletion of a critical exon or by introduction of an indel (insertion/deletion) within the coding sequence of a critical exon (see picture below).



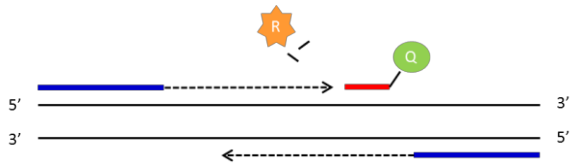


qPCR genotyping strategy

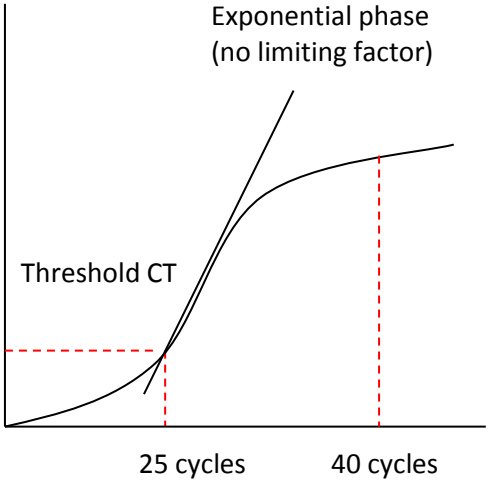
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that is complimentary to part of the DNA sequence between the forward and reverse primers. At the 5' end of the probe there is a fluorescent reporter (R) and at the 3' end a quencher (Q). Whilst they are in close contact with each other there is no fluorescent signal.



As the forward primer is extended the reporter is cleaved from the probe resulting in a fluorescent signal being detected. Once the primer extends enough to release the quencher this signal is blocked. By using probes with different fluorescent signals multiple PCR assays can be multiplexed and run together.



PCR reaction plot



The number of cycles the PCR takes to reach a set threshold is known as the CT value. This is inversely correlated to the amount of template DNA in the sample.

e.g. CT 25 = 2 x template DNA
CT 26 = 1 x template DNA
CT above 30 = no template represented in the sample

CT value can be used to determine how many copies of a particular allele samples have.

All our qPCR are run in duplicate. A FAM labelled genotyping assay is run in multiplex with a VIC labelled internal control Dot1l.

F13A1-L35V Genotyping Strategy

Samples are genotyped with WT and Mutant assays. These are FAM labelled assays that are designed to detect the critical exon that has been modified. If the animal contains the modified allele the copy number of the WT assay should drop by 1 and Mutant assay increase by 1 copy. For autosomal genes that have been targeted this means the following

WT= 2 copies of the WT assay and 0 copies of the Mutant assay
HET = 1 copy of the WT assay and 1 copy of the Mutant assay
HOM = 0 copies of the WT assay and 2 copies of the Mutant assay

F13A1-L35V-WT1 assay (FAM labelled probe)

TCTCCCTTCCCAAGGATCCAGTAAAGCTGAGAATGTCAGATACTCCAGCAAGCACCTTTGGGGGGAGGCGAGCAG
TCCCGCCCAATAACTCCAATGCTGCAGAAGTGGACCTCCCAACTGAGGAGCTACAAGGCCTGGTGCCAAGGGGT
GTCAACCTGAAAGGTAGGAGAAGCATACCACTGTGTCTACTAGAGCCGGACCTCATTTCCCATGAGGGATCTGTG

F13A1-L35V-WT1 primers and probe

Primer 1 = TCCCGCCCAATAACTCCAATG
Primer 2 = ACCTTTCAGGTTGACACCCCT
Probe = CAAGGCCTGGTGCCAAGG

F13A1-L35V-DONOR2-MUT1 assay (FAM labelled probe)

TCTCCCTTCCCAAGGATCCAGTAAAGCTGAGAATGTCAGATACTCCAGCAAGCACCTTTGGGGGGAGGCGAGCAG
TCCCGCCCAATAACTCCAATGCTGCAGAAGTGGACCTCCCAACTGAGGAGCTACAAGGCGTGGTGCCCAGGGGT
GTCAACCTGAAAGGTAGGAGAAGCATACCACTGTGTCTACTAGAGCCGGACCTCATTTCCCATGAGGGATCTGTG

F13A1-L35V-DONOR2-MUT1 primers and probe

Primer 1 = ACACCCCTGGGCACCAC
Primer 2 = TCCCGCCCAATAACTCCAATG
Probe = TAGCTCCTCAGTTGGGAGGTCCA

Dot1l internal control (VIC labelled)

TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTCGTTTTCT
GTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCTGGGGCAAGGCTTT
ATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCCACACCCATCAG

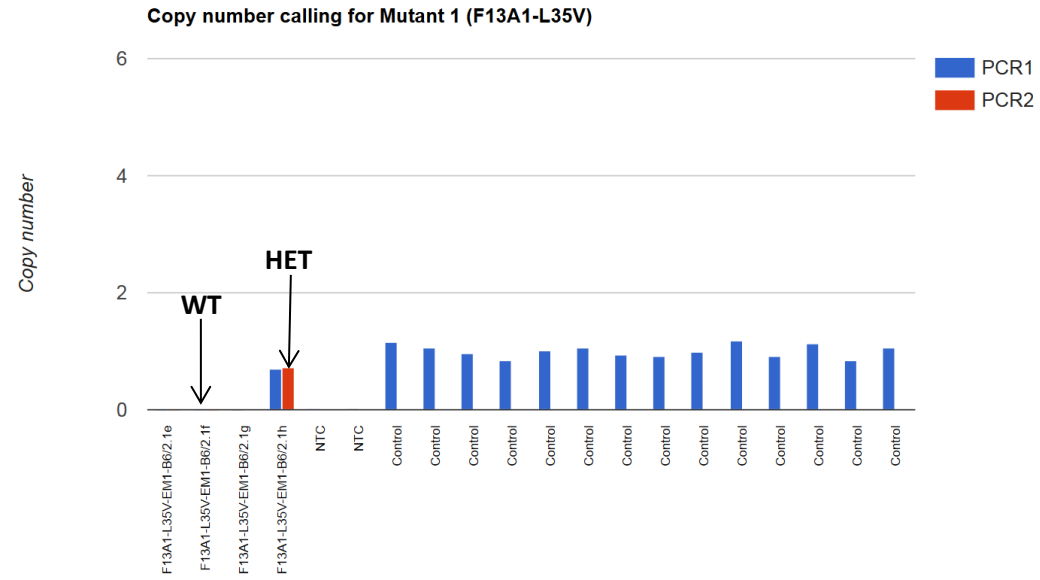
Primer 1 = GCCCCAGCACGACCATT
Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

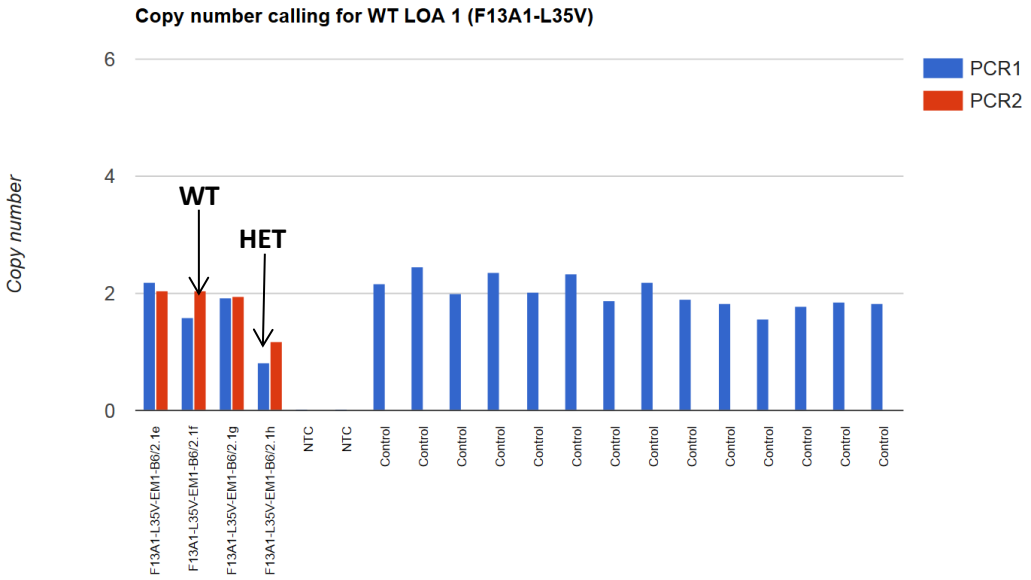
qPCR master mix

ABI GTX Taqman master mix	5µl
Primers Dot1L_2F (20µM)	0.225µl
Primers Dot1L_R (20µM)	0.225µl
Probe DotL_2M (5µM)	0.2µl
FAM Assay (probe 5µM & primers 15µM each)	0.3µl
ddH20	1.55µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

F13A1-L35V LOA copy called result, image showing both replicates and controls for both WT and Mutant assays

From T184161





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Approved by:	Debbie Williams

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Characterisation of a novel genetically modified FXIII-A L34V model shows altered thromboembolism dynamics

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BACKGROUND: Coagulation factor XIII (FXIII) is a key enzyme that stabilises blood clots by crosslinking fibrin molecules together, as well as crosslinking fibrinolysis inhibitors into fibrin. The human FXIII-A V34L sequence variant leads to increased activation rates, forming clots with thinner fibrin fibres and smaller pores, and is protective against thrombotic disease. However, the mechanism(s) by which V34L protects against thrombosis is unclear.
AIMS: To establish a murine FXIII-A V34L model and study its role in mechanisms of thromboembolic disease.
METHODS: FXIII-A 34V and FXIII-A 34L (wild-type) mice were weighed weekly for 12 weeks to compare growth. Plasma FXIII activation was assessed by biotin incorporation assay, and antigen levels by western-blotting. Whole blood clot formation, strength and lysis was measured by ROTEM (EXTEM, EXTEM+tPA, FIBTEM), providing clotting time (CT), maximum clot firmness (MCF) and lysis time (LT). Whole blood clot contraction and erythrocytes extrusion (haemoglobin) were measured for 2h, before the final clot weight was quantified. For pulmonary and cerebral embolism models, mice were anaesthetised and injected with AlexaFluor647-fibrinogen before injuring the inferior vena cava and carotid artery, respectively, with FeCl3 for 3 minutes. After 57 minutes, the mice were perfused-fixed and FITC-albumin (in gelatin) was injected in the circulation. Lungs or brains were harvested, dehydrated, optically cleared and imaged with a light-sheet microscope visualising both FITC-fluorescing vasculature and AlexaFluor647-fluorescing emboli. Image stacks were used to reproduce a 3D image of an organ and emboli larger than 50 μm3 were quantified using IMARIS software.
RESULTS: No significant difference in animals' growth was observed between the groups. FXIII-A 34L plasma showed a 40.4% increase in activation rates compared to 34V, similar to comparison with human variants. No difference in plasma FXIII-A and fibrinogen antigen levels was observed between the groups. ROTEM studies showed no difference between the groups for CT, MCF and LT, whether in the presence (EXTEM) or absence (FIBTEM) of functional platelets. The clot volumes and serum haemoglobin levels were not significantly different at each time-point between the groups, and the final clot weight was similar. However, 34L mice showed a 45.8% decrease in cerebral emboli count compared to 34V mice, while there was no significant difference in pulmonary embolism data between the two mouse strains.
CONCLUSIONS: The murine FXIII-A 34L variant increased activation rates over the 34V variant comparable to that of the human variant (+68%), despite similar circulating levels. No differences were observed between the variants in *ex-vivo* studies of whole blood clotting, however *in-vivo* studies point to decreased cerebral embolism profiles for mice homozygous for the FXIII-A 34L variant. These results indicate that by altering embolism dynamics FXIII-A 34L variant could possess protective capabilities in the cerebral circulation.