***1. Genotyping of R1.40 transgenic mice***

DNA was isolated from 2 mm tail snips and two genotyping techniques were performed: standard PCR followed by gel electrophoresis on 1.5% agarose gel, and the TaqMan® allelic discrimination assay (Applied Biosystems, Foster City, CA, USA).

*1.1. DNA isolation*

DNA was isolated from mouse tissue as described in the literature (Truett, et al., 2000). In brief, tissue was obtained through tail snipping (2 mm) and was placed in Eppendorf tubes followed by adding 75µl of a mix of 25 mM sodium hydroxide (NaOH) and 0.2 mM ethylenediaminetetraacetic acid (EDTA). The tubes were placed in a thermocycler (Bio-Rad, Hercules, CA, USA) at 98°C for 1 hr followed by cooling at 15 ºC. After that, 75µl of 40 mM Tris hydrochloride (HCl) of pH 5.5 was added and mixed. Tubes were centrifuged at 1700 × g for three minutes and supernatants were collected. DNA concentration and quality was checked using the NanoDrop 2000 Micro-Volume UV-Vis Spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

*1.2. Standard PCR and agarose gel electrophoresis*

Standard PCR genotyping method for R1.40 mice was performed as provided by the Jackson laboratory (The Jackson Laboratory, Bar Harbor, ME, USA). The primer pairs for the APP transgene used were: IMR6938 (5'-CTT CAC TCG TTC TCA TTC TCT TCC A-3') and IMR6939 (5'-GCG TTT TTA TCC GCA TTT CGT TTT T-3'). Each reaction consisted of a mixture of 10µl TaqMan® genotyping master mix (Applied Biosystems, Foster City, CA, USA), 2µl of each primer and 2µl DNA from test samples. PCR amplification step was performed using a thermocycler (Bio-Rad, Hercules, CA, USA) as follows: 94°C for 3 min and then 35 cycles of

94°C for 30 sec, 65°C for 30 sec and 72°C for 30 sec, and then at 72°C for 2 min. Bands were then separated by gel electrophoresis on 1.5% agarose gels containing ethidium bromide and were visualized using Typhoon™ 9410 Variable Mode Imager (GE Healthcare, Piscataway, NJ, USA).

*1.3. TaqMan® allelic discrimination assay*

To distinguish between hemizyougs and homozygous R1.40 YAC APP and WT, TaqMan® allelic discrimination assay was performed. SNP genotyping assay, catalogue: M_23312848_20, containing the context sequence ([VIC/FAM]): TAC TGC TCA CTC TTT TAA CAA ACA T[A/G]G GAA ACT AAT GAA ATG GTA TCA CAA was obtained from ABI (Applied Biosystems, Carlsbad, CA, USA) and the assay was performed according to ABI protocol. Briefly, each reaction consisted of 6.25µl 2× TaqMan® genotyping master mix (Applied Biosystems, Foster City, CA, USA), 0.625µl 20× SNP genotyping assay mix, 3.125µl RNase free water and 2.5µl genomic DNA from test samples. The pre-read run and PCR amplification were conducted in a 7500 Real-Time PCR System following ABI standard protocol, which was 95°C for 10 min followed by 40 cycles of 92°C for 15 sec and 60°C for 1 min. Following the post-read run, results were analyzed using the sequence detection software (SDS, version 1.3) from ABI and the allelic discrimination scatter plots were automatically generated.

**Results**

*Genotyping of R1.40 mice*

The primer pairs IMR6938 and IMR6938 amplified the 84 bp PCR product from the mutant human APP whereas no PCR product was expected from the non-transgenic mice (Fig. 1A). Thus, the detected bands at 84 bp in agarose gels represent either a hemizygous or homozygous

mutant APP alleles. Allelic discrimination scatter plots were generated showing three cluster groups classifying the unknown samples as hemizygous APP mice having both allele A and allele G, and as homozygous APP mice having allele G or wild type mice having only Allele A (Fig. 1B). Thus, by allelic discrimination assay we were able to identify hemizygous and homozygous transgenic APP mice, and the wild type mice as well.

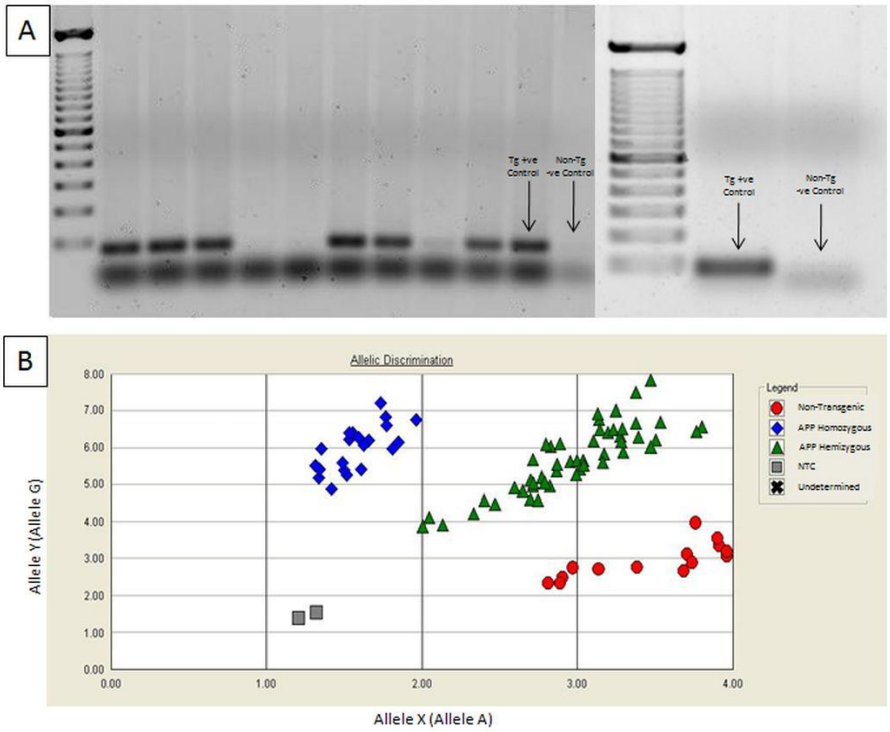


**Figure 1. Genotyping of in-house bred APP YAC transgenic mice, line R1.40**. Genetic identification and allelic discrimination was performed using standard protocols (see methods section). **A)** Genetic identification of YAC APP transgenic (Tg) and non-transgenic (non-Tg) mice using standard PCR protocol; **B)** A scatter plot of allelic discrimination analysis.

### *2. Determination of target preference in probe trials*

Analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons was performed to determine target preference of different mice groups in the conducted probe trials of the Morris water maze (MWM).

Our results indicate that in the first probe trial on Day 1 following the last day of daily training sessions, both WT and R1.40 mice groups spent significantly more time in the target quadrant ($F(3,68) = 5.421$, $p = 0.0021$; $F(3,72) = 5.535$, $p = 0.0018$, respectively) (Fig. 2A). However, in the second probe trial conducted on Day 11 following the last day of daily training sessions, the WT group retained preference to the target quadrant while the transgenic R1.40 group failed to do so ($F(3,68 = 2.93$, $p = 0.0397$; $F(3,72) = 0.9626$, $p = 0.4155$) (Fig. 2B), indicating poor memory retention in the second probe trial.

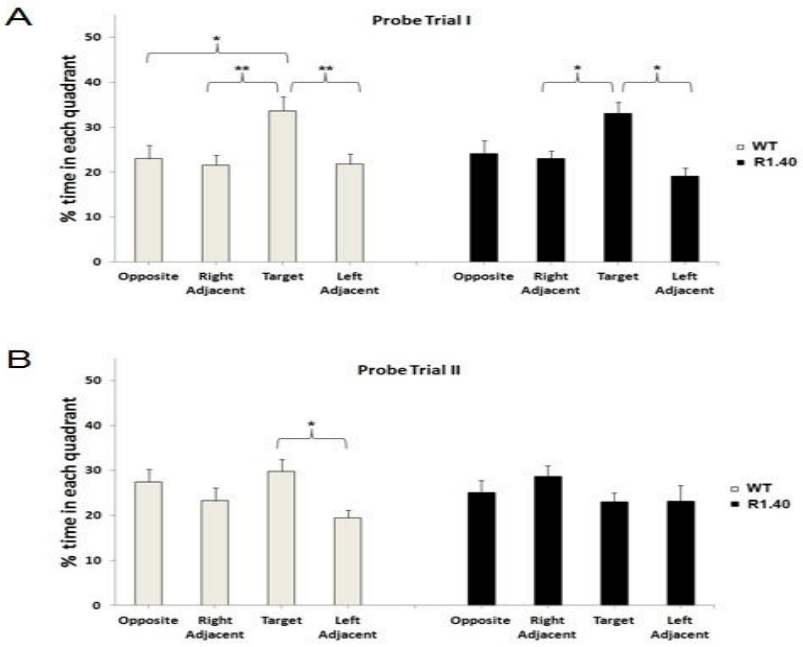


**Figure 2. Target preference of mice during the probe trials of MWM**. Comparisons of percent time spent in different quadrants are shown for MWM probe trial I conducted on Day 1 (A) and probe trial II (B) conducted on Day 11 following the last day of the daily training sessions. "*" Indicates that values are significant as determined by ANOVA analysis with Tukey-Kramer post-hoc test to compare all pairs of columns ($*p<0.05$, $**p<0.01$), obtained using GraphPad InStat 3 software. WT n=18; R1.40 n=19.

Following treatment with 5 and 50 mg/kg/day of tolfenamic acid or vehicle, two probe trails on Day 1 and 11 following the last day of training sessions were conducted. ANOVA analysis revealed that mice group treated with 50 mg/kg/day showed significant preference for the target quadrant in both probe trial I (Fig. 3A) and in probe trial II (Fig. 3B), ($F(3,24) = 4.096$, $p = 0.0176$; $F(3,24) = 3.179$, $p = 0.044$, respectively), indicating memory improvement following treatment with tolfenamic acid.

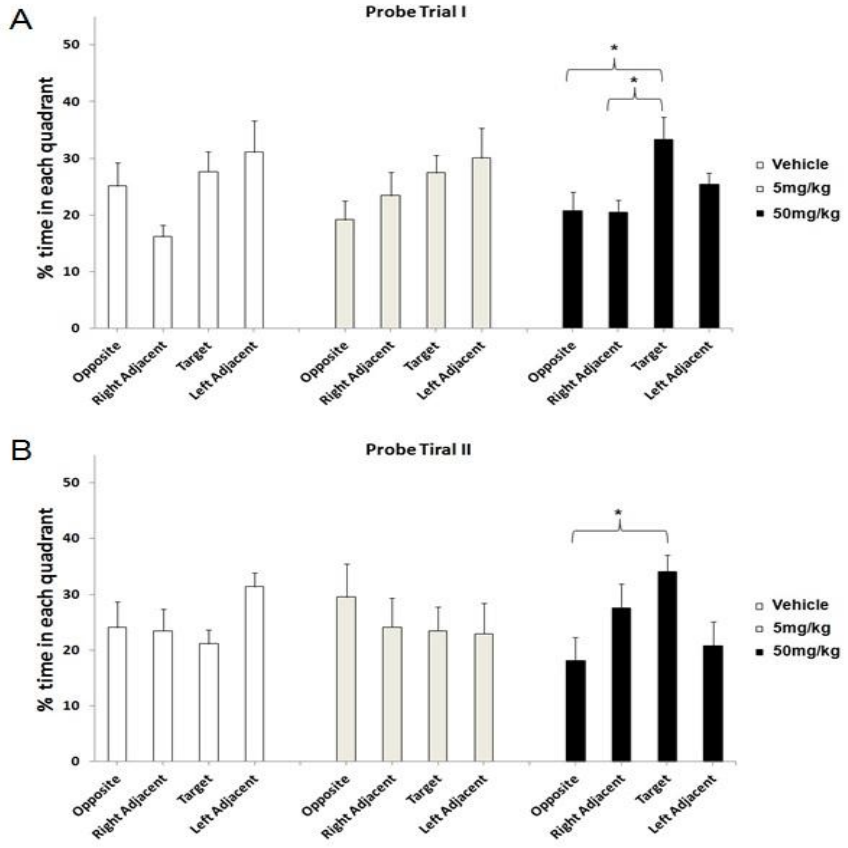


**Figure 3. Target preference during the probe trials of MWM following tolfenamic acid treatment**. Comparisons of percent time spent in different quadrants are shown for different treatment groups for MWM probe trial I (A) conducted on Day 1 and probe trial II (B) conducted on Day 11 following the last day of the daily training sessions. "*" Indicates that values are significant as determined by ANOVA analysis with Tukey-Kramer post-hoc test to compare all pairs of columns ($*p<0.05$), obtained using GraphPad InStat 3 software. Vehicle n=6; 5 mg/kg/day n=6; 50 mg/kg/day n=7.