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**14. ABSTRACT**

In year 3, our third investigator continued to repeat the experiments, testing our hypothesis that mouse genetic backgrounds affect the outcome of Rbpj deletion-induced regression of brain arteriovenous malformation (AVM), or BAVM, in the Notch4* mouse model. We had made time-consuming efforts to restore the genetic background of the first investigator and had obtained promising preliminary data to demonstrate the regression seen by the first investigator. We plan to submit the manuscript as soon as we repeat our finding that deletion of Rbpj blocks brain AVM formation. We also re-analyzed the DAPT data and re-designed a new study, with 3 times higher dose but much shorter duration (3 days) DAPT treatment to achieve the goal of testing the efficacies of DAPT in prevention or treatment BAVM formation in the Notch4* mouse model of the disease. We have also completed majority of the ephrin-B2 deletion studies as proposed and as reported in year 2. Our results revealed surprising finding that deleting ephrin-B2 in brain endothelial cells, either arterial or venous endothelial had little effects on brain vascular structure. However, deleting ephrin-B2 in all and venous, but not arterial, endothelium in the whole body leads to chylothorax, illness, and early moribund. Based on these results, we generated the *Tie2-tTA;TRE-Notch4*;Slco1c1-CreER$^{T2}$;ephrin-B2$^{fx/fx}$* mutant mice, deleting ephrin-B2 in the endothelium in the brain but not in other organs

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## 1. INTRODUCTION:

This project addresses the PRMRP Topic Area on *Vascular Malformations*. Our long-term goal is to develop novel therapeutics to ameliorate arteriovenous malformation (AVM), a severe vascular malformation in which arteries carry blood directly to veins through enlarged vessels, known as AV shunts, bypassing capillaries and thus failing to deliver necessary nutrients to tissues. These AV shunts easily rupture, leading to hemorrhage. AVMs pose a high risk to military personnel, as they affect young people, often males, a population highly represented among active military personnel. AVMs can occur anywhere in the body, but brain AVM (BAVM) is the most dangerous form of the disease. BAVMs are only treated by risky neurosurgery or radiotherapy, and potentially lead to life-threatening strokes. The cellular and molecular bases of BAVM pathogenesis remain unknown, limiting the rational design of molecular interventions. A deeper understanding would inspire new discoveries in diagnostic tools, prevention strategies, and pharmacological treatments, improving prognosis and care for patients in both military and civilian populations. Our central hypothesis in this study is that intermediary proteins in the signaling pathway governed by the cell surface protein Notch may serve as new therapeutic targets for BAVM treatment. We and others have linked abnormalities in Notch signaling to AVMs and we have reported that the Notch pathway is hyperactive in BAVM patients. Introducing a constantly active mutant of Notch, called Notch4*, in mice results in BAVMs in 100% of animals. Built on our strong background and preliminary data, we propose herein to test our hypothesis that inhibition of Rbpj or ephrin-B2, two proteins in the Notch signaling pathway, also causes the regression of Notch4*-induced BAVMs, demonstrating that BAVMs can be treated by a molecular intervention. We also plan to test a drug that has been tested for Alzheimer's disease therapy in humans, DAPT, which inhibits Notch activity. Further, we plan to use state-of-the-art two-photon imaging techniques from our Partner PI's laboratory to visualize disease processes in living animals in real time and provide the first clues on exactly how BAVMs regress after these three interventions. The proposed research promises new molecular targets and novel vessel-specific therapeutic strategies, ultimately leading to non-invasive treatments, diagnostics, and prevention strategies for BAVM and more broadly for AVMs and other vascular malformations at large.

## 2. KEYWORDS:

Brain arteriovenous malformation, arteriovenous malformation, Notch, RBPJ, ephrin-B2, arterial venous specification, vascular, two-photon imaging, mouse, endothelial cells, arteries, veins, dual antiplatelet therapy (DAPT), gamma secretase inhibitor

3. ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

**What were the major goals of the project?**

**List the major goals of the project as stated in the approved SOW.** If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.

| **Specific Aim 1: Determine the effects of inhibiting Rbpj on the regression of the Notch4* BAVM model.** | | | |
|---|---|---|---|
| **Major Task 1:** Determine the effect of deleting *Rbpj* in all endothelial cells on the cessation and regression of *Notch4**-induced BAVM | **Months** | **Site 1 PI Wang UCSF** | **Site 2 Schaffer Cornell** |
| Subtask 1: Generate mutant and control mice for *Rbpj* deletion using Cdh5(PAC)-CreER$^{T2}$ | Continue until publication | Ongoing | |
| Subtask 2: Evaluate mice for hallmarks of BAVM formation using imaging, histology, and clinical evaluation | 1-8 mos | More than 90% completion. Require further work until publication of the study. In the process of repeating the study. [Repeating the experiments. Performing Extensive further work. ] | |
| Subtask 3: Evaluate data using statistical analysis | 6-12 mos | More than 90% completion. Require further work until publication of the study. In the process of repeating the study. [Repeating the experiments. Reanalyze the data] | |
| **Major Task 2:** Determine the effect of deleting *Rbpj* from the venous and capillary endothelium on the cessation and regression of *Notch4**-induced BAVM | | | |
| Subtask 1: Generate mutant and control mice for venous *Rbpj* deletion using Apln-CreER$^{T2}$ | Continue until publication | Ongoing about 10% completed | |
| Subtask 2: Evaluate mice for hallmarks of BAVM formation using imaging, histology, and clinical evaluation | 5-12 mos | 10% | |
| Subtask 3: Evaluate data using statistical analysis | 12-14 mos | Dr. Wang | |
| **Major Task 3:** Determine the effect of deleting *Rbpj* from the arterial endothelium on the cessation and regression of *Notch4**-induced BAVM | | | |
| Subtask 1: Generate mutant and control mice for arterial *Rbpj* deletion using BMX(PAC)-CreER$^{T2}$ | Continue until publication | Ongoing about 10% completed | |
| Subtask 2: Evaluate mice for hallmarks of BAVM formation using imaging, histology, and clinical evaluation | 12-20 mos | Dr. Wang | |
| Subtask 3: Evaluate data using statistical analysis | 18-22 mos | Dr. Wang | |
| **Major Task 4:** Determine the effect of blocking Notch with DAPT on the cessation and regression of *Notch4**-induced BAVM | | | |
| Subtask 1: Generate mutant and control mice for *Notch4** and treat with DAPT via intraperitoneal injection | Continue until publication | Completed studies with oral gavage delivery. [Re-designing new experiments] | |

| | | | |
|---|---|---|---|
| Subtask 2: Evaluate mice at P36 for hallmarks of BAVM using imaging, histology, and clinical evaluation | 16-22 mos | 90% Completed. | |
| Subtask 3: Analyze data for attenuation of BAVM symptoms | 18-22 mos | 90% Completed. | |
| Subtask 4: Evaluate mice after termination of DAPT treatment using imaging, histology, and clinical evaluation for return of BAVM | 18-30 mos | 90% Completed | |
| Subtask 5: Analyze data for termination effects using statistical analysis | 24-30 mos | 90% Completed | |
| Milestone: Local IACUC Approval | Prior to project start | Completed | |
| Milestone: ACURO Approval | 4 mos | Completed | |
| Milestone: Establish effects of *Rbpj* deletion on BAVM regression | 20 mos | Completed, pending further work until publication of the study. In the process of repeating the study. | |
| Milestone: Establish efficacy of DAPT in treatment of BAVM | 30 mos | 90% Completed | |
| **Specific Aim 2: Determine the roles for *ephrin-B2* as a therapeutic target in the *Notch4** BAVM model.** | | | |
| **Major Task 1:** Determine the temporal effect of endothelial deletion of *ephrin-B2* on BAVM progression | | | |
| Subtask 1: Generate mutant and control mice for endothelial *ephrin-B2* deletion using Pdgfb(PAC)-CreER$^{T2}$ | Continue until publication | Ongoing | |
| Subtask 2: Evaluate mice for hallmarks of BAVM formation using imaging, histology, and clinical evaluation | 6-18 mos | About 90% completed | |
| Subtask 3: Evaluate data using statistical analysis | 10-18 mos | About 90% completed | |
| **Major Task 2:** Determine the effect of deleting *ephrin-B2* in the arterial endothelium on AVM progression | | | |
| Subtask 1: Generate mutant and control mice for arterial *ephrin-B2* deletion using BMX(PAC)-CreER$^{T2}$ | Continue until publication | About 90% completed | |
| Subtask 2: Evaluate mice for hallmarks of BAVM formation using imaging, histology, and clinical evaluation | 26-32 mos | About 90% completion | |
| Subtask 3: Evaluate data using statistical analysis | 30-32 mos | About 90% completion | |
| **Major Task 3:** Determine the effect of deleting ephrin-B2 in the venous and capillary endothelium on AVM progression | | | |
| Subtask 1: Generate mutant and control mice for venous and capillary *ephrin-B2* deletion using Apj-CreER$^{T2}$ | Continue until publication | Ongoing | |
| Subtask 2: Evaluate mice for hallmarks of BAVM formation using imaging, histology, and clinical evaluation | 26-32 mos | About 90% completion | |
| Subtask 3: Evaluate data using statistical analysis | 30-32 mos | About 90% completion | |
| **Major Task 4:** Determine the effect of deleting *ephrin-B2* from venous and capillary endothelium on the prevention and treatment of *Notch4** mediated BAVMs | | | |
| Subtask 1: Generate mutant and control mice for venous and capillary *ephrin-B2* deletion in *Notch4** mice using Apj-CreER$^{T2}$ | Continue until publication | Ongoing | |
| Subtask 2: Evaluate mice for BAVM regression using imaging, histology, and clinical evaluation | 28-36 mos | About 90% completion | |
| Subtask 3: Evaluate data using statistical analysis | 34-36 mos | About 90% completion | |
| Milestone: Establish effects of *ephrin-B2* deletion on BAVM regression | 36 mos | About 90% completion | |
| **Specific Aim 3: Reveal the mechanism of AVM regression by time-lapse live imaging in the *Notch4** BAVM model.** | | | |
| **Major Task 1:** Imaging cellular dynamics of *Notch4**-induced BAVM regression after venous and capillary deletion of *Rbpj* | | | |

| | | | |
|---|---|---|---|
| Subtask 1: Establish working imaging technique amenable to subsequent 5D imaging | 1-12 mos | | Dr. Schaffer |
| Subtask 2: Generate mutant and control mice for venous and capillary *Rbpj* deletion in *Notch4** mice using Apj-CreER$^{T2}$ | Continue until publication | Ongoing | |
| Subtask 3: Use 5D imaging through cranial window to observe cerebrovasculature before and after *Rbpj* deletion | 12-24 mos | Dr. Wang | Dr. Schaffer |
| Subtask 4: Evaluate data using statistical analysis | 18-24 mos | | Dr. Schaffer |
| **Major Task 2:** Imaging cellular dynamics during BAVM regression after venous and capillary endothelial deletion of *ephrin-B2* | | | |
| Subtask 1: Generate mutant and control mice for venous and capillary *ephrin-B2* deletion in *Notch4** mice using Apj-CreER$^{T2}$ | 24-36 mos | 10% | |
| Subtask 2: Use 5D imaging through cranial window to observe cerebrovasculature before and after *ephrin-B2* deletion | 24-36 mos | Dr. Wang | Dr. Schaffer |
| Subtask 3: Evaluate data using statistical analysis | 30-36 mos | | Dr. Schaffer |
| Milestone: Establish structural and cellular effects of *Rbpj* deletion on BAVM regression | 24 mos | | |
| Milestone: Establish structural and cellular effects of *Rbpj* deletion on BAVM regression | 24 mos | | |
| Milestone: Establish structural and cellular effects of *ephrin-B2* deletion on BAVM regression | 36 mos | | |

## What was accomplished under these goals?

**Aim 1: Determine the effects of inhibiting *Rbpj* on the regression of the Notch4* BAVM model.** *We hypothesize that genetic deletion of Notch signaling will lead to the regression of BAVMs.* We propose to determine the effects of deleting Notch signaling mediator *Rbpj* in all endothelium on the regression of the BAVMs (Aim 1.1) and to delineate arterial vs. venous contributions during BAVM regression by deleting *Rbpj* selectively in arterial (Aim 1.2) or venous (Aim 1.3) endothelium. *We also hypothesize that pharmacological inhibition of Notch signaling will lead to the regression of BAVMs.* We proposed to determine the efficacy of Notch inhibitor DAPT on the regression of *Notch4**-induced BAVMs by IP injection (Aim 1.4).

***Aim 1.1 Determine the effect of deleting* Rbpj *in all endothelium on the cessation and regression of* Notch4*-*induced BAVM.***
We have continued our time-consuming efforts to overcome complications in this study. As reported in year 1, we had obtained comprehensive data, leading to the significant finding that endothelial deletion of Rbpj reversed brain AVM formation. However, as reported in year 2, to ensure high reproducibility, a second investigator repeated the experiments done by the first investigator in year 1. The second investigator's reults showed less reduction in brain AVM formation in Rbpj deleted vs not deleted mice, when compared to the first investigator's results. Given the discrenpencies, the second investigators kept trying to repeat the experiments but was unable to obtain similar results as the first investigator. Both investigators had done multiple the experiments, thus we believe both investigators' results were true. After extensive analysis, comparision, and discussions, we identified that these two investigators were using mice with the same genotypes but in different genetic backgrounds. We reasoned that the differential mouse genetic background in their studies might contribute to the descrenpencies in the outcomes. We thus developed the hypothesis that differential genetic backgrounds led to different AVM formation and regression. We have started to test the genetic background of the first investigator used, which was several generations in FVBN, to repeat the results of the first investigator. To this end, by year 2, we had backcrossed the mice from mixed background into 3 generations into FVBN background (called FVBN-N3 mice). In year 3, we produced more of the FVBN-N3 mice in order to perform the experiments and had started to test the AVM formation, as well as the AVM regression after RBPJ deletion in AVM mice in this FVBN-N3 background. In terms AVM formation, we found that the phenotype is similar to the first investigator's. This means that we are in the right direction. In terms of testing the effect of AVM regression after RBPJ deletion, we unfortunately did not obtain the mutants with all alleles to perform this tests, despite long efforts of screening many offsprings. Part of this low yield was due to lost of homozygousity in backcross to FVBN background. Part of the low yield was likely due to the infection outbreak in our mouse facility, and during the infection control, quarantine, and recovery, we had mouse breeding and

production issues. It has been time and effort consuming, and these complications have delayed our progresses, but we have been continuing our efforts to repeat the results and to publish our finding. In addition to performing the experiments, we continued to prepare the manuscript and generate the figures needed for the reports.

***Aim 1.2*** ***Determine the effect of deleting*** **Rbpj** ***from the*** **venous and capillary endothelium** ***on the cessation and regression of*** **Notch4*-*induced BAVM.**

*Rationale*: We have previously reported that activation of *Notch4** in the arterial endothelium alone is not sufficient to induce BAVM [10]. Thus, *Notch4** expression in the non-arterial endothelial compartment is required for the onset/formation of BAVM in *Notch4** mice. *We hypothesize here that blocking canonical* Notch4 *signaling specifically in the venous endothelium will mitigate features of* Notch4*-*induced BAVM.* We will use Apj-CreER$^{T2}$ to delete *Rbpj* from venous and capillary ECs in postnatal *Notch4** mice. As shown in our preliminary data, Apj-CreER$^{T2}$ is specifically active in venous and most capillary ECs in the brain (Fig. 8B).

This subaim proposes studies to delineate the role of deleting Rbpj in venous and capillary endothleial cells in limiting *Notch4**-induced BAVM, which is built on the finding from the studies proposed in Aim 1.1. We thus needed to focus on our effort for the studies in Aim 1.1.

***Aim 1.3*** ***Determine the effect of deleting*** **Rbpj** ***from the arterial endothelium on the cessation and regression of*** **Notch4*-*induced BAVM.**

*Rationale*: We have previously shown that while pan-endothelial deletion of *Rbpj* at P7 blocks the formation of BAVM in *Notch4** mice, arterial-endothelial deletion of *Rbpj* does not [10]. Thus, arterial *Rbpj* is not required for the formation of BAVM in *Notch4** mice. *We hypothesize that arterial* Rbpj *is likewise not required for BAVM maintenance/progression and that arterial-endothelial deletion of* Rbpj *will not attenuate features of* Notch4*-*induced BAVM.* Though we predict no effect on BAVM, these experiments are crucial to delineate the contributions to BAVM pathogenesis by the arterial vs. venous endothelial compartments and to define an effective target site for BAVM treatment. We will use arterial-endothelial Cre, BMX(PAC)-CreER$^{T2}$, to delete *Rbpj* from ECs in postnatal *Notch4** mice. We have previously demonstrated that BMX(PAC)-CreER$^{T2}$ activity is restricted to the arterial endothelium in the neonatal mouse brain (Fig. 8A).

This subaim includes studies to delineate the role of deleting Rbpj in arterial endothleial cells in limiting *Notch4**-induced BAVM, which is built on the finding from the studies proposed in Aim 1.1. We thus needed to focus on our effort for the studies in Aim 1.1.

***Aim 1.4*** ***Determine the effect of blocking Notch with DAPT on the cessation and regression of*** **Notch4*-*induced BAVM.**

*Rationale*: As sustained *Notch4** expression is required to maintain BAVM, *we hypothesize that pharmacological inhibition of Notch signaling mitigates* Notch4*-*induced BAVM*. We will administer DAPT (Calbiochem/Millipore EMD), which inhibits *Notch4** activation and examine effects on the onset and severity of BAVM in *Notch4** mice. *We also hypothesize that continued DAPT treatment is not necessary to repress* Notch4*-*induced BAVM.* Since turning on *Notch4** after P21 does not lead to BAVM, we reason that after BAVM regression by DAPT treatment, even if *Notch4** activity returns without continuing DAPT treatment, BAVM will not re-appear, so long as DAPT treatment is halted after P21. Our pilot experiment demonstrated effective blockage of Notch4 activation via DAPT, as measured by reduced Notch4-ICD and Cx40 expression in ECs (Fig. 9).

In year 2, we had completed the proposed study of daily DAPT IP injection at 30mg/kg body weight From P16 until P36 showing that this DAPT regiment did not, to our surprise, lead to reduction in AVM formation. This negative result was not what we expected. Because a negative result is not as informative in reporting the finding. Based on the prior knowledge, our original hypothesis was that DAPT treatment would lead to AVM regression. In year 3, we had been further pursuing this study, continued the analysis, literature search, discussions with experts, and optimalizaion in experimental design. We found that in young mice doses as high as 100mg/kg body weight, but short treatment duration of 3 days, have been reported. We had designed a study to test if a high dose (100mg/kg body weight) for 3 days under 5D live time-laspe imaging would allow us to reveal if DAPT treatment can lead to AVM regression. This will be major undertaken, we are carefully examing the feasibility before initiating this study.

**Aim 2: Determine roles for *ephrin-B2* as a therapeutic target in the *Notch4** BAVM model.**

*We hypothesize that the increase in* ephrin-B2 *expression in the venous branch of* Notch4* *mice is critical for AVM progression and that suppression of* ephrin-B2 *in the venous branch will lead to BAVM regression.* We will examine the temporal effects of *ephrin-B2* inhibition throughout endothelium on BAVMs (Aim 2.1). Next, we will examine spatial specificity by deleting *ephrin-B2* in the

arterial (Aim 2.2) or venous (Aim 2.3) endothelium. We predict that venous deletion, targeting where the gene is not normally expressed, will not lead to BAVMs. Finally, we will delete *ephrin-B2* from veins of *Notch4** mice already developed BAVMs, blocking the *Notch4**-induced ectopic *ephrin-B2* to elicit the regression of existing AVMs (Aim 2.4). Together, these experiments will reveal the site-specific effect of *ephrin-B2* on *Notch4**-induced BAVM and suggest that venous *ephrin-B2* deletion, particularly beyond the neonatal stage, after BAVMs are already established, is a novel treatment strategy.

***Aim 2.1* Determine the temporal effect of endothelial deletion of ephrin-B2 on BAVM progression.**

*Rationale*: *ephrin-B2* is a downstream target of Notch, and endothelial deletion of *ephrin-B2* during development leads to AV defects similar to those caused by endothelial expression of Notch4*. *We hypothesize that deletion of* ephrin-B2 *from postnatal endothelium at birth leads to features of BAVM.*

As reported in Year 2, we have generated a superior mouse model with brain endothelial cell-specific deletion of *ephrin-B2* using the *Slco1c1-CreER$^{T2}$* mice, or *Slco1c1-CreER$^{T2}$;ephrin-B2$^{fx/fx}$* mice, to avoid systemic and peripheral defects seen in *Pdgfb(PAC)-CreER$^{T2}$* mediated deletion. In year 3, we had completed the phenotypic characterization of *Slco1c1-CreER$^{T2}$;ephrin-B2$^{fx/fx}$* model and found no detectable abnormalities in this mouse model. To ensure the reprodubilities of the finding, more than one investigator performed the analysis. This result is significant in two accounts. First, *ephrin-B2$^{f/f}$* is not required in brain endothelium or the *Slco1c1-CreER$^{T2}$* active cells. Second, this mouse model would allow us to delete *ephrin-B2* in our AVM model.

***Aim 2.2* Determine the effect of deleting ephrin-B2 *in the arterial endothelium on AVM progression.***

*Rationale*: ephrin-B2 is strongly expressed in the endothelium of arterial branch of vessels, but not in the veins. Therefore, we reason that phenotypes caused by its pan-endothelial deletion (Aim 2.2) are due to its deletion from the arterial endothelium. *We hypothesize that arterial deletion of* ephrin-B2 *in the postnatal brain will phenocopy pan-endothelial deletion of* ephrin-B2.

In year 3, we re-analyzed our data of arterial deletion of ephrinB2 using BMX(PAC)-CreER$^{T2}$ with the brain endothelial cell-specific deletion of *ephrin-B2* using the *Slco1c1-CreER$^{T2}$* mice. Since we did not detected any abnormalities in the brain or other parts of the body in the *Slco1c1-CreER$^{T2}$;ephrin-B2$^{fx/fx}$*, suggesting ephrin-B2 is not required in the brain endothelial cells. This finding is consistent with the finding that ephrin-B2 is not required in the arterial endothelial cells in the brain. Together these results lead us to conclude that ephrinB2 is not required in the endothelial cells, including arterial arterial endothelial cells in the brain.

***Aim 2.3* Determine the effect of deleting ephrin-B2 *in the venous and capillary endothelium on AVM progression.***

*Rationale: ephrin-B2 is not normally expressed in the veins and capillaries. Therefore, we hypothesize that deletion of* ephrin-B2 *from the vein and capillary will not lead to BAVM.*

In year 3, we further verified with more experiments that venous deletion of ephrin-B2 using Apj-CreER$^{T2}$ resulted chylothorax and illness, reaching moribund similar to pan-endothelial deletion model, at an early age before P16. We have reproducibly observed these findings without detecting any obvious brain AVM in these mutants. This provocative finding further support venous endothelial ephrin-B2, and more broadly endothelial ephrin-B2, is not required for brain arterial venous structure or vascular morphology, but required in the separation of lympathic and blood systems in the lung.

***Aim 2.4* Determine the effect of deleting ephrin-B2 *from venous and capillary endothelium on the prevention and treatment of* Notch4*-*mediated BAVMs.***

*Rationale:* As BAVMs develop in *Notch4** mice, *ephrin-B2* expression increases in the brain capillaries and veins. When *Notch4** is turned off and BAVMs regress, *ephrin-B2* expression decreases. [7-9] This *Notch4**-dependent differential expression led us to hypothesize a functional role for *ephrin-B2* during BAVM pathogenesis. *We hypothesize that expression of* ephrin-B2 *is required in the capillary and vein for the formation and maintenance of Notch4*-induced BAVM and that deletion of* ephrin-B2 *from* Notch4* *venous and capillary endothelium will prevent BAVM formation and induce BAVM regression.*

As reported, in Year 2, we had successfully generated the *Tie2-tTA;TRE-Notch4*;Slco1c1-CreER$^{T2}$;ephrin-B2$^{fx/fx}$* mutant mice, deleting ephrin-B2 only in the brain endothelium to examine its effect in Notch4*-mediated brain AVM formation. In Year 3, we have been evaluating the effects of removing brain endothelial ephrin-B2 in brain AVM formation, and found that ephrin-B2 in brain endothelium was not required for Notch4*-mediated brain AVM formation, as without ephrin-B2, *Tie2-tTA;TRE-Notch4** mice developed AVM, illness, enlarged hearts, similar to mice with ephrin-B2. This is a novel, provocative finding, along with other data obtained in this Aim, would advance our knowledge on the role of ephrin-B2 in AVM formation and maintenance.

## What opportunities for training and professional development has the project provided?

We have 4 postdoctoral fellows, 1 graduate student, 2 recent collage graudates before graduate school, who have

worked on this project in the past year. I provide them with one-on-one training and mentoring. In addition, we have weekly group meeting where one postdoc presents and the entire group discusses and comments on his/her research progress, including experimental design, data interpretation, and future plans. We also have a bi-weekly journal club, where one postdoc presents a leading article and the group discusses and gives input. I also ask postdocs to submit weekly updates and I am available for any questions during the week. I also meet with my postdocs, as well as graduate student, and SRAs as needed, in which we analyze data, experimental design, and overall progress. I am also readily available as needed beyond these regular meetings. Postdocs are also encouraged to attend campus-wide Research In Progress meetings, retreats, and other forums as well as national and international conferences. Dr. Schaffer and members of his group also provided 2 photon microscopy trainees to my group. UCSF Animal Care and other Core facility, as well as collaborators also provide hands on technical trainings. Together, these engagements provide many opportunities for professional development for our trainees.

## How were the results disseminated to communities of interest?

We will submit our manuscripts to report our finding.

## What do you plan to do during the next reporting period to accomplish the goals?

We plan to continue the project as proposed and as outlined in the above “achievement” section.

4**. IMPACT**: *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

**What was the impact on the development of the principal discipline(s) of the project?**

First, our data in this period continue support our finding that deleting Rbpj can induce brain AVM regression in the Notch4* mouse model of the disease. Second, DAPT treatment at the tested conditions did not led to obvious benefit in preventing or treatment brain AVM development in the Notch4* mouse model. Third, while arterial endothelial deletion of ephrin-B2 did not, whereas venous endothelial deletion did, phenotype pan-endothelial deletion in terms chylothorax, illness, and early moribund.

**What was the impact on other disciplines?**

Nothing to report

**What was the impact on technology transfer?**

Nothing to report

**What was the impact on society beyond science and technology?**

Nothing to report

**5. CHANGES/PROBLEMS:**

Nothing to report

**Changes in approach and reasons for change**

No change in this report period.

**Actual or anticipated problems or delays and actions or plans to resolve them**

- Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

**Changes that had a significant impact on expenditures**

- Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

- Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

**Significant changes in use or care of human subjects - NA**

**Significant changes in use or care of vertebrate animals.- No**

**Significant changes in use of biohazards and/or select agents - No**

**6. PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

**Publications, conference papers, and presentations**
*Nothing to Report*

**Website(s) or other Internet site(s)**
*Nothing to Report*

**Technologies or *techniques***

*Nothing to Report*

**Inventions, patent applications, and/or licenses**

—

*Nothing to Report*

**Other Products**
*Nothing to Report*

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### What individuals have worked on the project?

| | |
|---|---|
| Name: | Rong Wang |
| Project Role: | PI |
| Researcher Identifier: | |
| Nearest person month worked: | 6 |
| Contribution to Project: | She directed the project, trained and supervised other researchers. She designed mouse genetic approaches and breeding, closely guided experiments and data analysis. She coordinated efforts with the Partnering PI and ensured achieving proposed goals timely. |
| Funding Support | NIH/NINDS |

| | |
|---|---|
| Name: | Songchang Chen |
| Project Role: | Professional Researcher |
| Researcher Identifier: | |
| Nearest person month worked: | 1 |
| Contribution to Project: | He learnt to perform Notch4 immunostainging, pmouse genetic breeding, genotyping, immunostaining |
| Funding Support | NIH/NHLBI |

| | |
|---|---|
| Name: | I-Ju Lee |
| Project Role: | Graduate student and Staff Reaearch Associate |
| Researcher Identifier: | |
| Nearest person month worked: | 0.4 |
| Contribution to Project: | He provided critical two photon imaging support, including two-photon microscope maintenance and training |
| Funding Support | NIH/NINDS |

| | |
|---|---|
| Name: | Maya Aslam |
| Project Role: | Staff Reaearch Associate/post graduate researcher |
| Researcher Identifier: | |
| Nearest person month worked: | 1 |
| Contribution to Project: | She learnt and performed the mouse breeding, genotyping, and mouse colony management. Provided research support and lab managerial duties, including equipment and facility |

| | |
|---|---|
| | maintenance, ordering supply, and compliance. |
| Funding Support | NIH?NINDS, Foundation |

| | |
|---|---|
| Name: | Kayla Branyan |
| Project Role: | Post Doc |
| Researcher Identifier: | |
| Nearest person month worked: | 2 |
| Contribution to Project: | She learnt and performed mouse genetics, breeding, genotyping, and colony management. Leant and performed experiments analyzing mouse model of brain AVM, including behavior, illness, gross pathology, vascular imaging, and phenotypic analysis |
| Funding Support | NIH/NHLBI |

| | |
|---|---|
| Name: | Xitao Wang |
| Project Role: | Post Doc |
| Researcher Identifier: | |
| Nearest person month worked: | 4 |
| Contribution to Project: | He learnt and performed mouse genetics, breeding, genotyping, and colony management. Leant and performed experiments analyzing mouse model of brain AVM, including behavior, illness, gross pathology, vascular imaging, and phenotypic analysis |
| Funding Support | NIH/NINDS |

| | |
|---|---|
| Name: | Shang Li |
| Project Role: | Post Doc |
| Researcher Identifier: | |
| Nearest person month worked: | 2 |
| Contribution to Project: | He provided training for two-photon imaging, including *in vivo* two-photon cerebrovascular |

| | |
|---|---|
| | imaging through cranial window, maintained mouse lines, provided cerebrovascular analysis expertise. |
| Funding Support | NIH/NINDS |

| | |
|---|---|
| Name: | Kota Kurisu |
| Project Role: | Post Doc |
| Researcher Identifier: | |
| Nearest person month worked: | 3 |
| Contribution to Project: | Learnt, performed, and trained cranial window surgeries, brain vascular imaging including casting. Collaborated in blood vessel diameter and blood flow velocity imaging and analysis, provided neurosurgical clinical perspectives. Participated in arterial deletion of RBPJ project. |
| Funding Support | Intramural, Foundation, NIH/NINDS, NIH/NIAMS |

| | |
|---|---|
| Name: | Spencer Thirtyacre |
| Project Role: | Post Doc |
| Researcher Identifier: | |
| Nearest person month worked: | 8 |
| Contribution to Project: | Learnt and performed the mouse breeding, genotyping and mouse colony management. Provided research support and lab managerial duties, including equipment and facility maintenance, ordering supply, and IACUC/EH&S compliances. |
| Funding Support | NIH/NHLBI |

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Yes, please see below the other support.

**What other organizations were involved as partners?**

Nothing to Report

## Other Support

**Wang, Rong**

**ACTIVE**

Title: RBPJ and EphrinB2 as molecular targets to treat brain arteriovenous malformation in Notch4- induced mouse model
Time Committed: 1.20 calendar months
Role: PI
Supporting Agency: DOD
Name of Contact: Sherry M. Apperson
Address: 820 Chandler Street, Fort Detrick, MD 21702
Performance Period: 09/30/2016 – 09/29/2020
Level of Funding: $1,783,125
Goals:

- Aim 1: Determine the effects of inhibiting Rbpj on the regression of the Notch4* BAVM model
- Aim 2: Determine roles for ephrin-B2 as a therapeutic target in the Notch4* BAVM model.
- Aim 3: Reveal the mechanism of BAVM regression by time-lapse live imaging in Notch4* mice.

Overlap: None

Title: Molecular pathogenesis of brain arteriovenous malformation
Time Commitment: 0.12 calendar months
Role: PI
Supporting Agency: NIH/NINDS
Name of Contact: Jim Koenig, Ph.D.
Address: 6001 Executive Boulevard, Room 2107, Rockville, MD 20852
Performance Period: 12/01/2009-03/31/2020
Level of Funding: $224,718 / year
Goals: The goal of this study is to determine eNOS pathway as a mechanism for the onset and progression of brain arteriovenous malformation in Notch-mediated mouse models of the disease.

- Aim 1: Determine the effect of endothelial Notch4* on venous endothelial dysfunction and BAVM formation.
- Aim 2: Determine the effect of endothelial Notch deficiency on arterial endothelial dysfunction and BAVM formation.
- Aim 3: Compare Notch and Alk1 mouse mutants in DA and CV development and BAVM formation.

Overlap: None

Title: Molecular Pathogenesis and Therapy for Critical Limb Ischemia
Time Committed: 2.28 calendar months
Role: PI
Supporting Agency: UC Tobacco Related Disease Research Pgm
Name of Contact: TRDRP Program Officer
Address: 300 Lakeside Drive, 6th Floor Oakland, CA 94612
Performance Period: 07/01/2018 – 06/30/2021
Level of Funding: $311,801 / year
Goals:

- Aim 1: Characterize the beneficial effects of Notch4* expression driven by arterial BMX-CreERT2 in aged mice recovering from EFAO.
- Aim 2. Examine vasodilation as a mechanism of Notch4*-mediated superior recovery after EFAO.
- Aim 3. Examine arteriogenesis as a mechanism of Notch4*-mediated superior recovery after EFAO.

Overlap: None

Title: Test AGNHW for brain hemorrhage in Notch4* mouse model.
Time Committed: 1.2 calendar months
Role: PI
Supporting Agency: Beijing Tong Ren Tang Chi. Med Co., Ltd
Name of Contact: Wu Wei
Address: 3 Dai King Street Tai Po Industrial Estate, New Territories, Hong Kong
Performance Period: 12/15/2018-12/14/2020
Level of Funding: $140,592/ year
Goals:

- Aim 1. Document the survival and illness in Notch4* mice treated with AGNH.
- Aim 2. Quantify hemorrhage in Notch4* mice treated with AGNH.
- Aim 3. Examine brain blood vessel structure in Notch4* mice treated with AGNH.
- Aim 4. Determine if AGNH prevents or attenuates changes in neurological behavior in Notch4* mice.

Overlap: None

Title: Development of an inhibitory antibody as a molecular therapy for brain arteriovenous malformation
Time Committed: 0.48 calendar months
Role: PI
Supporting Agency: UCSF Catalyst Awards Program
Name of Contact: Roopa Ramamoorthi
Address: UCSF CTSI, Box 0558 550 16th Street, Floor 6 San Francisco, CA 94143
Performance Period: 06/01/2018 – 05/31/2019
Level of Funding: $35,000/ year
Goals: Assess if inhibitory antibody to Notch is a valid therapeutic strategy for brain arteriovenous malformation.
Overlap: None

Title: Molecular Regulators of Hereditary Hemorrhagic Telangiectasia In a Novel Mouse Model
Time Committed: 0.60 calendar months
Role: PI
Supporting Agency: American Heart Association
Name of Contact: April Ciesla
Address: 7272 Greenville Avenue, Dallas, TX 75231-4596
Performance Period: 07/01/2019-06/30/2022
Level of Funding: $100,000 / year
Goals:

- Establish a novel HHT2 brain AVM mouse model by Alk1 gene deletion and to investigate additional trigger important for HHT2 brain AVM formation.

Overlap: None

**PENDING**

Title: Molecular Pathogenesis of Hereditary Hemorrhagic Telangiectasia
Time Committed: 3.6 calendar months
Role: PI
Supporting Agency: NIH/NINDS
Name of Contact: Jim Koenig, Ph.D.
Address: PO Box 6021 Rockville, Maryland 20852
Performance Period: 02/01/2020-01/31/2025
Level of Funding: $435,219 / year
Goals:

- Aim 1. Establish a novel HHT2 BAVM mouse model with brain endothelial specific Alk1 deletion.
- Aim 2. Investigate mechanisms of BAVM development in mice with brain endothelial specific Alk1
- Aim 3. Identify transcriptional targets of Alk1 in brain endothelial cells with genome-wide expression

profiling.
Overlap: None

**PREVIOUS**

Title: Notch signaling in mouse arterial-venous specification
Role: PI
Supporting Agency: NIH/NHLBI Name of Contact: Diane Reid, MD
Address: 6701 Rockledge Dr., Rockledge II, Room 7160, MSC-7926, Bethesda, MD 20892-7926 Performance Period: 04/01/2005-02/29/2017
Level of Funding: $243,787 / year
Goals: The goal of this study is to understand the role of Notch and related pathways in the molecular mechanisms underlying the embryonic development of parallel artery vein pairs in developing early mouse embryos.

- Aim 1: Examine Vascular Endothelial Growth Factor (VEGF)-mediated cell differentiation as a mechanism underlying heterogeneous arterial- and venous-fated ECs in the primordial DA and CV.
- Aim 2: Examine cell segregation as a mechanism to sort venous-fated ECs in the pDA to the pCV.
- Aim 3: Determine the role of Notch signaling in coordinating the development of parallel artery and vein pairs.
- Aim 4: Determine the requirement of endothelial Notch1 and COUP-TFII in AV specification of adult parallel artery and vein pairs.

Overlap: None

Title: Effects of endothelial deletion of Notch in AVM formation
Time Commitment: 0.5 calendar months
Role: PI
Supporting Agency: American Heart Association Name of Contact: Susan Mokhtari
Address: 7272 Greenville Ave., Dallas TX, 75231
Performance Period: 07/01/2013-06/30/2016
Level of Funding: $63,636 / year
Goals: The goal of this study is to evaluate whether mice lacking both Notch1 and Notch4 receptors in the endothelium develop brain arteriovenous malformation.

- Aim 1: Evaluate vascular pathology in mice lacking both Notch1 and Notch4 in the endothelium.
- Aim 2: Evaluate the mechanisms underlying the vascular pathology in mice lacking Notch in the endothelium.

Overlap: None

8. **SPECIAL REPORTING REQUIREMENTS**

**NA**

**9. APPENDICES**: NONE