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TITLE: Authentic Mouse Model of PRSS1-Related Hereditary Pancreatitis

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CONTRACTING ORGANIZATION: University of California, Los Angeles, CA

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14. ABSTRACT

Inroduction. The most frequent cause of hereditary pancreatitis is the p.R122H mutation in the serine protease 1 (PRSS1) gene, which encodes human cationic trypsinogen. This mutation renders trypsinogen resistant to protective degradation by chymotrypsin and thereby results in elevated intrapancreatic trypsin activity that elicits pancreatitis.
Methods. We introduced the p.R123H mutation, which is analogous to human PRSS1 mutation p.R122H, in the mouse cationic trypsinogen (isoform T7) and characterized the severity of experimentally induced acute pancreatitis in the novel *T7R123H* mice.
Results. The *T7R123H* knock-in mouse strain developed no spontaneous pathology in the pancreas or elsewhere. When pancreatitis was induced experimentally by cerulein injections, *T7R123H* mice exhibited similar intrapancreatic trypsin activation and disease severity as C57BL/6N control mice treated in the same manner. Sustained stimulation with cerulein, however, resulted in more severe chronic pancreatitis in *T7R123H* mice than in C57BL/6N controls.
Conclusion. The observations indicate that *T7R123H* mice exhibit more severe experimental pancreatitis in case of persistent pancreatic injury. The findings are consistent with the proposed pathogenic role of the p.R122H trypsinogen mutation in humans.

15. SUBJECT TERMS
None listed.

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1. Introduction

The most frequent cause of hereditary pancreatitis is the p.R122H mutation in cationic trypsinogen (PRSS1). This mutation renders trypsinogen resistant to protective degradation by chymotrypsin and thereby results in elevated intrapancreatic trypsin activity. High intrapancreatic trypsin levels drive pancreatitis onset and progression. Although PRSS1-related hereditary pancreatitis is a relatively rare disease, it is one of the best understood forms of human pancreatitis at the mechanistic level and thus it represents a paradigm for this potentially severe inflammatory disorder. Therefore, understanding how PRSS1 mutation p.R122H initiates pancreatitis should offer essential insight into the pathogenesis of all forms of human chronic pancreatitis. In our work funded by this grant award, we generated a novel mouse model of PRSS1-related hereditary pancreatitis. The *T7R123H* mice carry the p.R123H mutation in mouse cationic trypsinogen (isoform T7). Mutation p.R123H in mouse cationic trypsinogen is analogous to mutation p.R122H in human cationic trypsinogen. Characterization of the *T7R123H* mice with respect to pancreatitis has been ongoing, and accomplishments during the first reporting period have been reported previously.

This report is our second annual report covering the accomplishments between 03/01/2021 and 02/28/2022. Please note that the original PI of the grant, Dr. Zsanett Jancso, has left UCLA, and the project was taken over and continued by the new PI, Dr. Miklos Sahin-Toth as of 08/31/2021. Despite the ongoing COVID19-related restrictions, supply chain issues, and the PI change on the grant, progress in the second reporting period has been satisfactory, as detailed below.

2. Keywords: pancreas, trypsinogen activation, digestive protease, cerulein, acute pancreatitis, chronic pancreatitis, hereditary pancreatitis, preclinical mouse model

3. Accomplishments

What were the major goals of the project?

Specific Aim 1: Generate new knock-in mouse strain with mutated T7 cationic trypsinogen in the C57BL/6 background, carrying mutation p.R123H, which correspond to mutation p.R122H in human cationic trypsinogen (PRSS1).

Major Task 1: Generate knock-in strain with mutated T7 trypsinogen gene.

Major Task 2: Trypsinogen expression analyses.

Specific Aim 2: Experiments to assess spontaneous pancreatitis and increased susceptibility to experimental pancreatitis.

Major Task 3: Experiments to assess spontaneous pancreatitis.

Major Task 4: Investigation of susceptibility to experimental acute pancreatitis.

Major Task 5: Investigation of susceptibility to experimental chronic pancreatitis.

Major Task 6: Evaluation of intra-acinar trypsin activation.

What was accomplished under these goals?

Milestones Achieved: ***Major Tasks 1-4, and 6 have been successfully completed*** *and the results were reported in detail in our first annual technical report.*

Accomplishments during the second reporting period (Major Task 5)

Major Task 5: Investigation of susceptibility to experimental chronic pancreatitis. During the second reporting period we focused on Major Task 5, and evaluated whether acute pancreatitis in the *T7R123H* mice would progress to chronic pancreatitis more readily than in C57BL/6N mice. Given the small differences observed in acute pancreatitis severity in *T7R123H* mice, we reasoned that more sustained stimulation with cerulein may amplify the incipient pathology and result in overt disease. Therefore, we treated *T7R123H* and C57BL/6N mice with 8 hourly injections of cerulein on two consecutive days and euthanized the mice 3 days after the last injection. We found that cerulein-treated *T7R123H* mice generally developed more severe disease after an acute pancreatitis episode (Figure 1) while C57BL/6N mice completely recovered from the acute episode (not shown). The pancreatitis phenotype of *T7R123H* mice treated with this protocol was variable, with chronic pancreatitis, acute pancreatitis, and relatively intact pancreas histology observed in some mice (Figure 1). This type of the

inconsistency is also typical of human hereditary pancreatitis where age of onset and clinical presentation are variable.

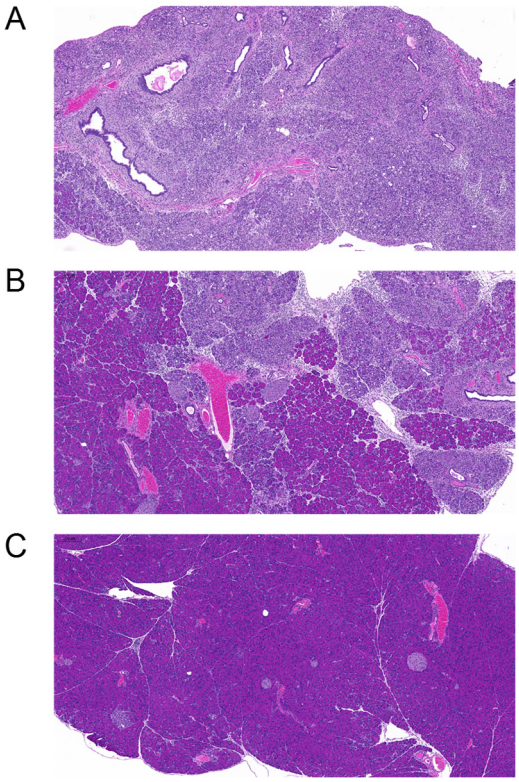

Figure 1. Histology of cerulein-induced chronic pancreatitis. C57BL/6N and *T7R123H* mice were given 8-times hourly cerulein injections for 2 consecutive days. Mice were euthanized 3 days after the last injection. Hematoxylin-eosin stained pancreas sections showing various degree of pathology. **A**, chronic changes were seen on 2/8 pancreas sections. **B,** Acute pancreatitis with chronic changes were seen on 3/8 pancreas sections. **C**, Relatively intact pancreas histology was seen on 3/8 sections.

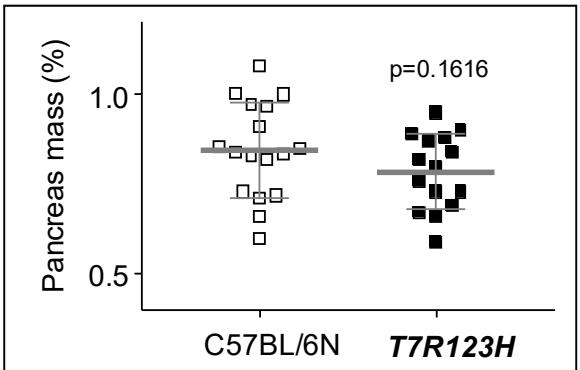

Figure 2. Pancreas mass (given as percentage of body mass) of C57BL/6N and *T7R123H* mice given 8-times hourly cerulein injections for 2 consecutive days. Mice were euthanized 3 days after the last injection.

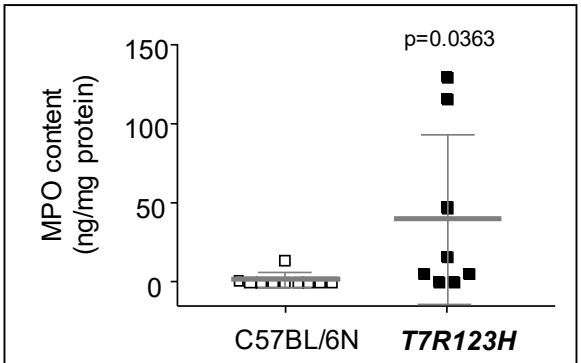

Figure 3. Myeloperoxidase (MPO) content of the pancreas from C57BL/6N and *T7R123H* mice given 8-times hourly cerulein injections for 2 consecutive days. Mice were euthanized 3 days after the last injection.

When the pancreas weight of a large number of mice was compared, there was a non-significant trend seen for smaller pancreas weight in *T7R123H* mice, suggesting some pancreatic atrophy (Figure 2).

Finally, we also measured MPO (myeloperoxidase) content in the pancreas, as a marker of acute inflammation, and we found variable levels, consistent with the histological pictures (Figure 3). Importantly, no MPO was detected in the pancreas of cerulein-treated C57BL/6N mice.

Milestone Achieved: A significant portion (80%) of Major Task 5 has been completed.

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

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the final period of the award, we will complete Major Task 5 by performing additional analyses in the chronic pancreatitis model described above. We will measure pancreatic hydroxyproline content to characterize fibrosis and intrapancreatic trypsin activity. We will also perform immunohistochemistry staining for the macrophage marker F4/80 and the ductal cell marker SOX9. The latter will be used to assess the extent of regenerative acinar-to-ductal metaplasia.

The most part of the final award period will be spent on completion of data analysis and publication of the results.

4. Impact

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

We generated a novel mouse model, which will offer conceptual proof for the role of trypsinogen mutations in hereditary pancreatitis. This model will allow the research community to study mechanistic details of the pathogenesis of hereditary pancreatitis. Furthermore, the novel mouse strain will be invaluable as a preclinical model for the testing of novel therapeutic and preventive approaches against pancreatitis.

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: Despite the ongoing COVID19-related restrictions, supply chain issues, and the PI change on the grant, progress in the second reporting period has been satisfactory.

6. Products: The *T7R123H* mouse model of hereditary pancreatitis.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	Zsanett Jancso, PhD
Project Role:	PI until 08-30-2021
Researcher Identifier (ORCID ID):	0000-0002-0572-5452
Nearest person month worked:	3.6
Contribution to Project:	Dr. Jancso has managed the project and performed the proposed experiments until her departure from UCLA.
Funding Support:	N/A

Name:	Miklos Sahin-Toth, MD, PhD
Project Role:	PI from 08/31/2021
Researcher Identifier (ORCID ID):	0000-0003-4513-9922
Nearest person month worked:	2
Contribution to Project:	Dr. Sahin-Toth took the project over from Dr. Jancso when she left UCLA
Funding Support:	N/A

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

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. Special Reporting Requirements: N/A

9. Appendices: N/A