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Modeling chronic pancreatitis as a complex genetic disease in mice

Zsanett Jancsó,

Alexandra Demcsák,

Miklós Sahin-Tóth*

Department of Surgery, University of California Los Angeles, Los Angeles, California, USA 90095

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To the Editor:

We read with great interest the recent articles by Wang et al. [1], Génin et al. [2], Sun et al. [3], and Németh et al. [4], in which the authors studied various genetic risk factors for chronic pancreatitis (CP). The ever increasing catalogue of risk genes and variants supports the long-held notion that CP is a complex genetic disorder. Thus, patients often carry multiple genetic alterations that interact in synergy and elicit disease onset and/or promote progression. To offer a conceptual framework for genetic risk in CP, we organized risk factors into mechanism-based schemes, which include the trypsin-dependent, misfolding-dependent, and ductal pathways [5].

More recently, novel mouse models harboring human pancreatitis-associated mutations emerged, providing strong evidence for the pathogenic nature of these risk variants [3, 6-10]. All mouse models of genetic CP published to date carry a single genetic alteration and none models CP as a complex genetic disease. We set out to fill this knowledge

*Correspondence to: Miklós Sahin-Tóth, 675 Charles E Young Drive South, MRL 2220, Los Angeles, CA 90095; Tel.: (310) 267-5905; msahintoth@mednet.ucla.edu.

CONTRIBUTORS

Study concept and design: ZJ, MST. Experiments: ZJ, AD. Acquisition, analysis or interpretation of data: all authors. Drafting of the manuscript: MST, AD. Critical revision of the manuscript for important intellectual content: all authors. Obtained funding: MST, ZJ. Administrative, technical or material support: all authors. Study supervision: MST. Final approval of manuscript as submitted: all authors. Guarantor of the article: MST.

COMPETING INTERESTS

The authors do not have competing interests.

ETHICAL APPROVAL

Animal experiments were performed at the University of California Los Angeles (UCLA) and at Boston University (BU) with the approval and oversight of the Animal Research Committee and the Institutional Animal Care and Use Committee, respectively, including protocol review and post-approval monitoring. The animal care programs at these institutions are managed in full compliance with the US Animal Welfare Act, the United States Department of Agriculture Animal Welfare Regulations, the US Public Health Service Policy on Humane Care and Use of Laboratory Animals and the National Research Council's Guide for the Care and Use of Laboratory Animals. UCLA and BU have approved Animal Welfare Assurance statements (A3196-01 and A3316-01, respectively) on file with the US Public Health Service, National Institutes of Health, Office of Laboratory Animal Welfare. Both institutions are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

gap by generating a novel strain carrying both the *T7K24R* trypsinogen mutant and the *Ctrb1-del* chymotrypsin deletion alleles. The *T7K24R* mice have the p.K24R mutation in mouse cationic trypsinogen (isoform T7), which is analogous to the human hereditary-pancreatitis associated p.K23R *PRSS1* mutation [9]. This mutation increases autoactivation of cationic trypsinogen and thus represents a gain-of-function variant. The *Ctrb1-del* mice are deficient in mouse chymotrypsins CTRB1 and CTRC [7, 8]. Chymotrypsins, primarily CTRC in humans and CTRB1 in mice, promote trypsinogen degradation and thereby protect against pancreatitis. The *Ctrb1-del* strain models the effect of loss-of-function chymotrypsin variants. Homozygous *T7K24R* or *Ctrb1-del* mice do not develop pancreatitis spontaneously, however, severity of cerulein-induced experimental pancreatitis is increased in both strains. Thus, the *T7K24R* and *Ctrb1-del* alleles do not cause pancreatitis but sensitize mice for the disease, in a manner that is reminiscent of the effects of human CP-associated risk variants.

To test whether the *T7K24R* and *Ctrb1-del* alleles would synergize and elicit spontaneous pancreatitis, we created mice homozygous for both alleles (*T7K24R* × *Ctrb1-del*). Remarkably, mice with the compound genotype developed severe, early-onset pancreatitis (Figures 1 and 2). Body weight of *T7K24R* × *Ctrb1-del* mice was noticeably reduced at 3, 4 and 6 weeks of age compared to the parent strains or C57BL/6N controls (Figure 1A), suggesting pancreatic insufficiency. Consistent with atrophic CP, the pancreas weight was markedly diminished in *T7K24R* × *Ctrb1-del* mice (Figure 1B), even after normalization to body weight (Figure 1C). Hematoxylin-eosin staining of pancreas sections confirmed end-stage CP in 4-6-week-old *T7K24R* × *Ctrb1-del* mice while pancreas histology of the parent strains (not shown) was normal, indistinguishable from C57BL/6N controls (Figure 2). From the 3-week-old *T7K24R* × *Ctrb1-del* mice (n=8) analyzed histologically, 3 were still unaffected, 2 had acute pancreatitis, and 3 had CP of varying severity. In contrast, histology of the 7 mice evaluated at 4 weeks (n=4) or 6 weeks (n=3) of age showed severe CP in all cases, suggesting that in this model the age of pancreatitis onset is around 3 weeks of age and a brief period of acute pancreatitis might precede chronic progression.

When the disease phenotype of *T7K24R* × *Ctrb1-del* mice is compared to those of the parent strains, the difference is striking and demonstrates how the pathogenic alleles amplify each other's effect. These observations offer the first line of experimental evidence that independent risk factors for CP, particularly those within the same mechanistic pathway, can synergize and provoke overt pancreatic disease. The experiments also demonstrate that modeling CP in mice as a complex genetic disorder is feasible and facilitates the study of interactions between various CP risk alleles.

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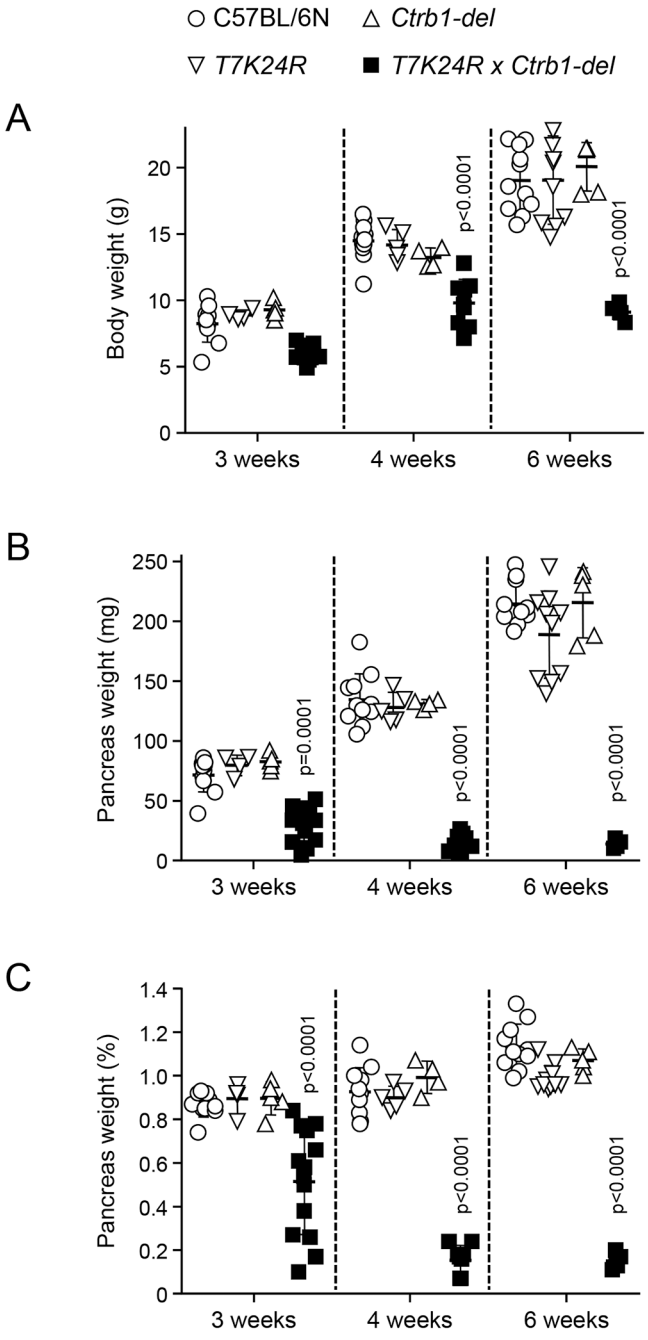

Figure 1.

Body weight and pancreas weight of *T7K24R* × *Ctrb1-del* mice at 3, 4, and 6 weeks of age. For comparison, age-matched data for C57BL/6N mice and the parent strains *T7K24R* and *Ctrb1-del* are also shown. **A**, Body weight. **B**, Pancreas weight in mg units. **C**, Pancreas weight as percent of body weight. Individual data points were graphed with the mean and SD indicated. The differences of means between the groups were analyzed by one-way ANOVA followed by Tukey's post-hoc test. $P < 0.05$ was considered statistically significant.

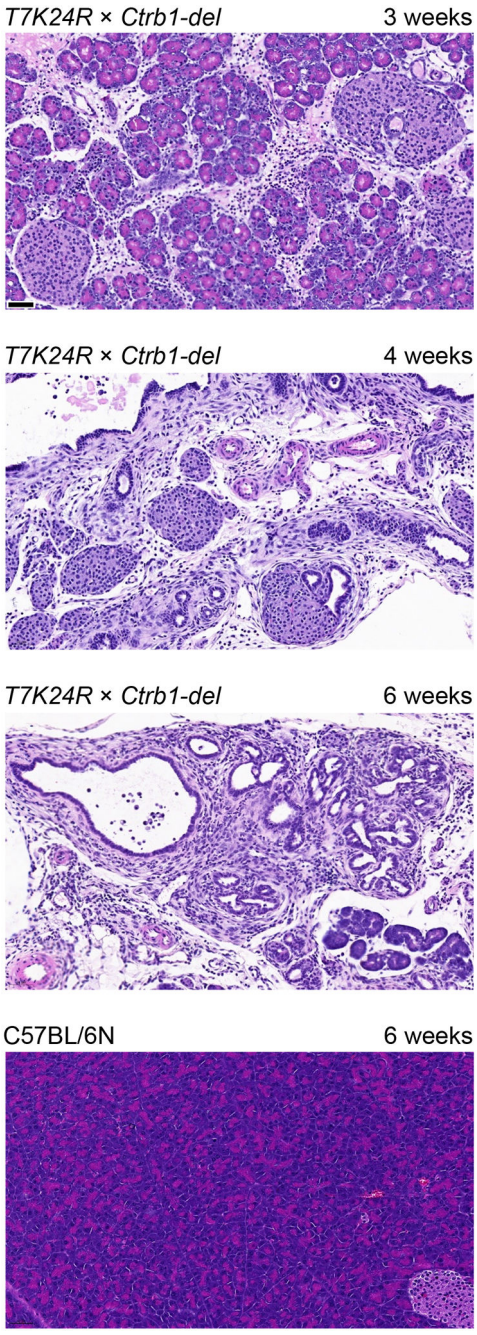

Figure 2.

Histology of the pancreas from *T7K24R* × *Ctrb1-del* mice at 3, 4, and 6 weeks of age. For comparison, pancreas section from a 6-week-old C57BL/6N mouse is shown. Representative hematoxylin-eosin stained pancreas sections are shown. The scale bars correspond to 50 μm. Note that the phenotype at 3 weeks of age was variable, see text for details. Although not shown, pancreas histology of 6-week-old *T7K24R* and *Ctrb1-del* mice was normal, indistinguishable from that of C57BL/6N controls.