siRNA therapy for TTR-related ocular amyloidosis

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Transthyretin (TTR)-related Familial amyloidotic polyneuropathy (FAP)

- FAP is characterized by amyloid deposition caused by amyloidogenic TTR (ATTR).
- Although ATTR is mainly produced by liver, ATTR is also produced by retinal pigment epithelium (RPE), choroid plexus, and pancreas.
- Ocular manifestations are commonly found and cause blindness.
Ocular manifestations after liver transplantation

Amyloid deposition on the fringed pupil

Vitreous opacity

Morphological change in the iris

Glaucoma

New therapeutic strategies to prevent the progression of ocular manifestations are urgently needed.

Unpublished Data

Liver transplantation: LT
Retinal laser photocoagulation is a common treatment used for diabetic retinopathy. This method could suppressATTR production by destroying the RPE.

Laser photocoagulation, which reduced the RPE, prevented the progression of amyloid deposition in the vitreous. Effective suppression of ATTR expression in the RPE may become a novel therapy for ocular amyloidosis.
Small interfering RNA (siRNA) therapy for ocular amyloidosis

TTR-specific siRNA

ATTR production in the RPE cells

RPE

TTR siRNA

ATTR

Ocular manifestations

Liver Cancer
Translational Excellence
Liver Cancer
Translational Excellence

TTR - specific siRNA

ATTR production

Amyloid deposition

No
Purpose

To evaluate the potential utilization of siRNA treatment as a novel therapy for ocular amyloidosis.
Materials and methods

siRNA (Alnylam): Human TTR, rat TTR- and control siRNA

*in vitro*: Human RPE cell line (ARPE-19)

*in vivo*: Dark Agouti (DA) rats
ATTR V30M transgenic (TG) rats

Evaluation of TTR expression:
real-time quantitative PCR

Ratio of ATTR V30M mRNA copies to rat GAPDH mRNA copies ($\times 10^{-3}$)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ratio</th>
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<tbody>
<tr>
<td>Liver</td>
<td>100,000</td>
</tr>
<tr>
<td>Brain</td>
<td>10,000</td>
</tr>
<tr>
<td>Eye</td>
<td>1,000</td>
</tr>
<tr>
<td>Lung</td>
<td>100</td>
</tr>
<tr>
<td>Kidney</td>
<td>10</td>
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</tbody>
</table>
Effect of human TTR siRNA on human TTR expression

< ARPE-19 >

Human TTR

Control siRNA
Human TTR siRNA
Rat TTR siRNA

human TTR/ human GAPDH mRNA relative expression

1 nM
10 nM
50 nM
Effect of rat TTR siRNA on endogenous rat TTR expression

< RPE from normal rats >

Endogenous rat TTR

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rat TTR/rat GAPDH mRNA relative expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treat</td>
<td>1.00</td>
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<tr>
<td>Saline</td>
<td>1.25</td>
</tr>
<tr>
<td>Control siRNA</td>
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<tr>
<td>Rat TTR siRNA</td>
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</tbody>
</table>

P < 0.01
Effect of human TTR siRNA on human ATTR expression

< RPE from ATTR V30M TG rats >

Human TTR/rat GAPDH mRNA relative expression

- No Treat
- Saline
- Control siRNA
- TTR siRNA

Human ATTR V30M
Summary

<In vitro>

• Human TTR siRNA specifically reduced human TTR mRNA in the RPE cell line
• No significant inflammatory responses were observed in the siRNA-transfected cells

<In vivo>

• Rat TTR siRNA significantly reduced endogenous rat TTR mRNA expression in the RPE
• Expression of ATTR mRNA was efficiently and specifically reduced by human TTR siRNA in the RPE
Future plans

- **Optimize siRNA delivery**
  - determine dose- and time-dependent effect
  - Utilize transfection reagents

- **Improve injection methodology**
  - subconjunctival space, subretina, etc.
  - increase volume of inoculum

- **Monitor for potential side effects**
  - Evaluation of inflammation, off-target effects, toxicity, etc.

A new therapy for TTR-related ocular amyloidosis
siRNA treatment may become a novel therapy for TTR-related ocular amyloidosis.