Gene therapy for Oculopharyngeal Muscular Dystrophy

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Oculopharyngeal Muscular Dystrophy (OPMD)

- Autosomal dominant disease: 1:100,000 in Europe, 1:1,000 in French/canadian population

- Typically onset occurs in the fifth to early sixth decade of life

- Phenotype characterized by:
  a) Progressive eyelid drooping
  b) Swallowing difficulties
  c) Proximal limb weakness

- Histology characterized by:
  - Decrease in fibre number
  - Variation in fibre size
  - Fibrosis
  - Intranuclear inclusions (INIs)
Mutation of PABPN1 leads to INIs

OPMD is due to expansion of the short (GCG) trinucleotide repeat in the coding sequence of the polyA binding protein nuclear 1 (PABPN1).

PABPN1: a ubiquitous protein that controls:
1) The length of mRNA poly(A) tails,
2) The mRNA export from the nucleus,
3) The alternative poly(A) site usage.

WT ATG (GCG)$_6$ (GCA)$_3$ GCG GGG GCT GCG..
MUT ATG (GCG)$_6$ (GCG)$_{2.7}$ (GCA)$_3$ GCG GGG GCT GCG...

12-17 ala instead of 10 $\rightarrow$ expPABPN1

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Intranuclear inclusions (INIs)
In skeletal muscle

- Resistant to degradation
- Trapping RNAs, proteins....wtPABPN1!!!

Tomé & Fardeau, 1980
Created with insertion of an expanded bovine PABPN1 driven by the human skeletal actin promoter

- Massive gene deregulation
- Severe muscle atrophy
- Mimics many pathological observations in human:
  - Progressive muscle weakness/ Atrophy/Fibrosis
  - Mitochondrial / Ubiquitin-Proteasome defects
  - All muscles contain INIs

Davies et al, Nature Medicine 2005
Trollet et al, Human Molecular Genetics 2010
Gene therapy approach

Impossible to specifically target expPABPN1

→ Suppression of endogenous PABPN1 (both normal and expanded)
→ Replacement with functional optPABPN1

IM Injection in Tibialis Anterior (TA) of 10-12 week old A17 mice

- AAV-shRNA3X (2.5x10^{10} vp/TA)
- AAV-optPABPN1 (1.3x10^{11} vp/TA)
- AAV-shRNA3X (2.5x10^{10} vp/TA) + AAV-optPABPN1 (1.3x10^{11} vp/TA)
- saline injection in TA of A17 and FvB mice as control
Effect on intranuclear inclusions

Treatment with KCl 1M to eliminate soluble aggregates

Malerba et al, Nat Comm 2017
GT treatment decreases fibrosis

Malerba et al, Nat Comm 2017
GT treatment improves muscle strength

**In situ**-muscle force measurement

- Increase in maximal force
- Normalization of specific maximal force to wild type level

Malerba et al, Nat Comm 2017
GT treatment normalises the transcriptome (Affymetrix analysis)

expPABPN1 expression in A17 mice causes extensive remodelling of muscle transcriptome


In A17 mice vs FvB, 865 transcripts were deregulated (FC>1.5; p<0.05)

Treatment with shRNA3X+optPABPN1 results in 98% “correction”

Malerba et al, Nat Comm 2017
From 2 AAVs to single BB-301 vector

Wildtype + Saline  A17 + Saline
A17 + BB-301 High Dose  A17 + BB-301 Low Dose

% Cells PABPN1 Intranuclear Inclusions

Mouse:  WT  A17  A17  A17
Treatment: Saline  Saline BB-301 BB-301
HD  LD
BB-301 Treatment Restores Muscle Force and Muscle Weight in A17 Mice

**Restoration of Muscle Force**

- **Wildtype Saline**
- **A17 BB-301 Low**
- **A17 BB-301 High**

**Restoration of Muscle Weight**

- Mouse: Wildtype, A17
- Treatment: Saline, BB-301 High Dose, BB-301 Low Dose

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**Graphs showing the restoration of muscle force and muscle weight with BB-301 treatment compared to control conditions.**
Conclusions

The gene therapy treatment:

1) Efficiently down-regulates expPABPN1 without affecting optPABPN1 expression
2) Abrogates insoluble intranuclear aggregates
3) Decreases fibrosis
4) Improves muscle strength
5) Completely recovers muscle mass (BB-301 vector)
6) Nearly normalizes the transcriptome (98% of gene expression is restored)
7) Single BB-301 vector shows great efficacy and allows clinical translation in human
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