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Appendix

Summary

Polyglutamine (polyQ) diseases are a group of nine neurodegenerative disorders, which are all caused by a CAG triplet repeat expansion, resulting in a gain of toxic polyQ protein function. The longer the CAG repeat, the earlier the disease manifestation and in most cases the disease onset is around midlife. Although the disorders have a monogenic cause and much research has been done the last decades, no therapies are available to cure or slow down the disease.

In **chapter 1** a short overview of the polyQ disorders and insight in the disease-causing polyQ proteins is given and their role in known disease mechanisms is described. The most prevalent and best studied polyQ disorders, being Huntington disease (HD) and spinocerebellar ataxia type 3 (SCA3), and the molecular biology of the disease causing proteins, huntingtin and ataxin-3, are extensively reviewed.

Furthermore, the opportunities for genetic therapies for polyQ disorders are discussed, focussing on what can be learned from other neurodegenerative disorders of which genetic therapies are in development or already used as therapy. Small molecules, called antisense oligonucleotides, are broadly used as potential treatment for neurodegenerative diseases. According to the specific chemical modifications and target binding site of the antisense oligonucleotide, they can either reduce expression or modify polyQ disease-causing proteins. Furthermore, the limitations and possibilities of the delivery of antisense oligonucleotides to the brain and into the affected neuronal cells are discussed.

While much research has been done on the underlying polyQ disease mechanisms, knowledge on regulation and expression of mRNA and protein expression are limited. **Chapter 2** describes subtle differences in huntingtin mRNA and protein expression in HD. In adult-onset HD less mutant huntingtin mRNA, but equal wild-type and mutant huntingtin protein levels are found. In juvenile HD subjects less mutant huntingtin protein compared to wild-type huntingtin protein is present. This indicates differences in huntingtin protein expression between adult-onset and juvenile HD.

In **chapter 3** CUG triplet-repeat antisense oligonucleotides are used to effectively reduce mutant polyQ transcript and protein levels in polyQ patient-derived fibroblasts. Although a reduction in wild-type CAG transcript levels was observed as well. This reduction was less pronounced than for the mutant transcript. Expression levels of other non-expanded CAG triplet repeat-containing transcripts investigated were not affected, verifying the specificity of the CUG triplet repeat antisense oligonucleotide for the mutant transcript. **Chapter 2** describes that the basal levels of mutant huntingtin mRNA and mutant huntingtin protein are equal or lower when compared to wild-type. This provides feasibility for genetic therapies like the CUG triplet-repeat antisense oligonucleotide that are not completely specific for the mutant huntingtin allele.

Next to specifically targeting the expanded CAG repeat transcripts to reduce the translation of mutant polyQ protein, **chapter 4** reports on removing the motifs that are implicated in the formation of toxic polyQ fragments. Soluble polyQ fragments, generated by proteolytic cleavage during the aggregation process, are considered to be the main toxic entities, resulting in neurodegeneration. In HD, removing a proteolytic cleavage site, implicated in the formation of huntingtin fragments containing the polyQ repeat has been shown to result in reduced toxicity. **Chapter 4** describes a potential therapeutic approach of preventing the formation of toxic huntingtin polyQ fragments by antisense oligonucleotides that induce skipping of exon 12 in huntingtin pre-mRNA. This antisense oligonucleotide-mediated protein modification resulted in a huntingtin protein lacking disease-implied cleavage motifs and subsequently reduced formation of toxic huntingtin polyQ fragments. This proof of concept shows a completely novel approach to reduce mutant huntingtin toxicity not by reducing expressing levels, but by modifying polyQ protein.

A more direct genetic approach to reduce polyQ-induced toxicity is shown in **chapter 5**. Here antisense oligonucleotide-mediated exon skipping is used to remove the toxic polyQ repeat from the ataxin-3 protein. Two different approaches are proposed with the same aim: removal of the CAG repeat-containing exon. Both approaches result in the formation of modified ataxin-3 protein that lacks the toxic polyQ repeat. The modified ataxin-3 protein lacks only a small part and maintains important wild-type functions of the protein.

The feasibility of the in **chapter 4 and 5** described antisense oligonucleotides was tested *in vivo* by injections of antisense oligonucleotides directly into the brain of mice. The *in vivo* and *in vitro* data described in this thesis suggest that both targeting the CAG repeat directly and antisense oligonucleotide-mediated exon skipping are promising potential therapeutic approaches to reduce polyQ-induced toxicity in HD, SCA3 and other polyQ disorders.

In **chapter 6** the main findings from this thesis are summarized. Recent developments are described and reflected how they relate to the results reported in this thesis. In addition, a reflection is given on how to get to clinical trials of genetic therapies like antisense oligonucleotide-mediated treatments for polyQ disease patients.

This thesis proposes various novel genetic therapies for polyQ disorders, aiming at reducing and/or modifying polyQ disease-causing proteins. Although extensive *in vitro* and *in vivo* research is important to rule out toxic off-target effects of the various antisense oligonucleotides and resulting modified proteins, these preclinical antisense oligonucleotide-mediated treatments look promising as therapeutic candidates for polyQ disorders.