Abstract

The causative role of the huntingtin (*HTT*) gene in Huntington's disease (HD) has been identified more than 25 years ago. The extension of CAG repeat stretch over 39 repeats in exon 1 of one *HTT* allele results in full penetrance of this neurodegenerative disorder. While the identification of the causative mutation raised hopes that development of the therapeutic compound will be easily achievable, the patients and their families are still waiting for treatment until now. The main reason for that might be the complex cellular function HTT that makes the determination of the pathologic mechanism difficult and the development of treatments even more challenging. Although a lot of different animal models have been generated until now, establishing a suitable model has still not been achieved yet. Due to its anatomy, physiology, and genetics, the minipig seems to be a suitable candidate for neurodegenerative disease models. Indeed, the existing Transgenic (Tg) Libechov minipig model manifests signs typical for HD in patients, but on the other hand significant inconsistencies have also been observed. The finding of malformation that partially shows the situation in human patients is true for both, the male reproductive tract as well as for the brain. The reason for this might be the fact the genetic modification is based on an additive transgene encoding a mutated and truncated cDNA construct and might, thus, not reflect numerous regulation processes that start from the initiation of transcription to the post-translation modification of the encoded protein. A better reflection of the human situation are Knock-In models which carry short part of human sequence, either the CAG mutated stretch alone or in combination with some short flanking sequences. There is, however, evidence that several regions outside the CAG stretch might influence the molecular consequences of a CAG stretch extension. The *in silico* prediction indicates strict specificity of most of these regions to the human gene, suggesting that the next generation of porcine HD model would require the humanization of the entire *HTT* locus. Given that large size of *HTT* locus and limitations in porcine cloning, such undertaking is a tremendous effort. Based on experience in mouse genetic modification, a Cre-mediated recombination would be the most efficient approach, but this requires introduction of lox sites into precise desired recombination points of the genome. The placement of such lox sites in the porcine genome was efficiently achieved by two different strategies, using either single-stranded short oligo-deoxynucleotides or extended bacterial artificial genome.