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Chapter 1 : Athena Diagnostics - DRPLA (ATN1) Repeat Expansion Test

CAG Repeat Expansion 85 Under some conditions of immunodetection, for instance, high concentration of antibody, very long exposure of the blot or low stringent washes, several additional.

The accumulation of polyglutamine-rich huntingtin proteins affects various cellular functions and causes selective degeneration of neurons in the striatum. Here, we demonstrate that the CAG repeat tract can be precisely excised from the HTT gene with the use of the paired Cas9 nickase strategy. The repeat excision inactivated the HTT gene and abrogated huntingtin synthesis in a CAG repeat length-independent manner. Because Cas9 nickases are known to be safe and specific, our approach provides an attractive treatment tool for HD that can be extended to other polyQ disorders. Introduction Expansions of short tandem repeat sequences in functionally unrelated genes are causative factors of numerous human hereditary neurological diseases. Currently, there are nine known neurodegenerative disorders caused by the expansion of CAG repeats within the coding regions of associated genes. Expanded polyglutamine polyQ protein may form intracellular aggregates and affects numerous cellular activities inducing pathogenesis via a gain of toxic function. Despite many years of research on an effective treatment method, HD and other polyQ diseases are incurable, and only their symptoms can be controlled. Several different strategies have already been employed in cellular and animal models of polyQ diseases to achieve the desired therapeutic effects Wild and Tabrizi, These strategies include the silencing of both HTT alleles in a non-allele-selective strategy and the targeting of single-nucleotide polymorphisms SNPs linked to repeat expansions. The repeat region itself may be targeted in an allele selective and non-selective manner Fiszer et al. Zinc finger nucleases ZFNs and transcription activator-like effector-based nucleases TALENs were the first tools that provided proof of principle for the idea of targeted inactivation of the expanded CAG repeats at a disease loci Mittelman et al. Double-strand breaks DSBs are repaired mainly by error-prone non-homologous end joining NHEJ , resulting in mutations that may cause frame-shifts in open reading frames, premature translation termination and transcript degradation by nonsense-mediated decay NMD. To increase specificity and reduce off-targeting, one of two cleavage domains in the Cas9 protein was mutated to act as a nickase Cas9n Cho et al. Nickases generate single strand breaks SSBs that are repaired with high fidelity. Therefore, the paired Cas9 nickase strategy can be useful in applications that require precise genome editing such as gene and cell therapy. Although this strategy is very promising, it requires a comprehensive analysis of the HTT gene haplotype structure. In this study, we examined paired Cas9 nickase strategy to inactivate the HTT gene by targeting sequences directly flanking the CAG repeat tract. We demonstrate that precise excision of the CAG repeats from the HTT gene results in the abrogation of protein synthesis in all investigated fibroblast cell lines derived from HD patients. Importantly, we also show that this specific and safe strategy leads to preservation of repeat-deficient transcript level, suggesting that the transcript may escape from NMD pathway. Ligated products were transformed into chemically competent E. The two-step PCR amplification program was used as follows: Cleavage products were separated in 1. Indel occurrence was estimated with an analysis of signal loss from the main PCR products. T7E1 analysis was performed as described for the HTT gene. After electrophoresis, the proteins were wet-transferred overnight to a nitrocellulose membrane Sigmaâ€”Aldrich. The primary antibodies, namely, anti-huntingtin 1: The protein bands were scanned directly from the membrane using a camera and quantified using Gel-Pro Analyzer Media Cybernetics. In addition, PCR products containing long stretches of repeated sequences may form various secondary structures e. Therefore, a determination of the exact indels frequency in this polymorphic, highly repetitive gene region was difficult using methods based on heteroduplex recognition by nucleases. Appropriate PAM sequences are highlighted in green. NLS, nuclear localization signal. The signal intensities of the two main bands marked with an asterisk was measured. Additional, faster migrating bands in samples non treated with T7E1 enzyme are secondary structure forms of the main product and their contribution is significantly reduced after denaturation of a sample directly before

gel electrophoresis see Figure S1. The lengths of the excised DNA fragments were between and bp for both alleles of the GM cell line, and bp for GM and and for the GM cell line. A shorter, bp PCR product was present in the three patients-derived cell lines; however, the editing efficiency was different, with the highest observed for the GM cell line. Interestingly, the level of the HTT transcript did not change in the cells treated with paired nickases, suggesting that the transcript may have escaped the nonsense-mediated mRNA decay pathway Figure 3B. The prematurely terminated translation product 43 amino-acid protein was not detected with the use of the N-terminal huntingtin antibody by western blot data not shown. Huntingtin inactivation via the Cas9 nickase pair in patient-derived fibroblasts. Plectin was used as the loading control. The lengths of the polyQ tracts in both alleles of the HTT protein in each cell line are marked with an arrow. In addition, the maximal cleavage efficiency of paired Cas9 nickases has previously been observed at sites with the tail-to-tail orientation separated by 10â€”30 bp Shen et al. In addition, the role of huntingtin in cell physiology and pathology is not fully understood Saudou and Humbert, , and therefore, strategies using selective silencing of the mutant allele alone and non-allele-selective silencing of both alleles are being developed in parallel. It has been shown using RNAi and antisense oligonucleotides that the knockdown of huntingtin, either the mutant or both mutant and normal is beneficial in mouse models of HD Harper et al. Stereotactic injection of AAVs expressing sgRNAs and SpCas9 into the striata of adult mice resulted in the depletion of huntingtin aggregates in the brain, thereby alleviating motor deficits and neuropathological symptoms. In our study, we present another repeat-depletion strategy to inactivate the HTT gene in which we further improve the approach by using a nickase version of Cas9 that is known to be more specific and safe than the wt Cas9. The efficiency of paired Cas9 nickase editing depends on the activity of two sgRNAs and the length of the target sequence between the two sgRNAs Mali et al. We confirmed the specificity and safety of the paired nickase strategy by testing selected off-target loci with T7E1 mismatch detection assays. The mechanism of this precise repeat excision and DNA repair without scars , atypical for NHEJ is poorly known and needs further studies. Even single DSB in the region flanking the repeated sequence was sufficient to generate clean loss of repeats. Interestingly, in our study the level of the shortened HTT transcript did not change, suggesting that the transcript may be NMD-resistant. Genome editing with the use of a more universal CAG repeat-targeting strategy is still challenging due to the lack of specific PAM recognized by targeted nucleases, off-targeting induced by sgRNA comprising repeats and problems with the selective inactivation of mutant alleles alone. Similar problems have already been overcome by antisense and RNAi technologies Hu et al. However, the in-frame shortening of the CAG repeat tract with the use of genome editing tools would be the most desired and universal approach and is our goal for future studies. Conflict of Interest Statement The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Supplementary Material The Supplementary Material for this article can be found online at:

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Chapter 2 : Genome Wide Screening of CAG Trinucleotide Repeat Lengths in Breast Cancer (pdf) | Paperi

Antibody-Based Detection of CAG Repeat Expansion Containing Genes In all the cases studied so far, the normal gene products tolerate a rather wide variation in size of a polygln tract (ranging typically between glns) without any detectable adverse effect.

Advanced Search Abstract To investigate whether the expansion of CAG repeats of the TATA-binding protein TBP gene is involved in the pathogenesis of neurodegenerative diseases, we have screened patients with various forms of neurological disease and identified a sporadic-onset patient with unique neurologic symptoms consisting of ataxia and intellectual deterioration associated with de novo expansion of the CAG repeat of the TBP gene. The mutant TBP with an expanded polyglutamine stretch 63 glutamines was demonstrated to be expressed in lymphoblastoid cell lines at a level comparable with that of wild-type TBP. The present study provides new insights into sporadic-onset trinucleotide repeat diseases that involve de novo CAG repeat expansion. Introduction Expansions of CAG trinucleotide repeats have been identified as the causative mutations in at least eight hereditary neurodegenerative disorders 1â€” Although the genes causing these disorders do not share homology except for the CAG repeats, there are several striking similarities among these disorders: Since the TBP gene contains particularly long and polymorphic CAG repeats ranging from 25 to 42, the TBP gene has been investigated intensively as a candidate for psychiatric disorders 14â€” In this study, we found a patient with unique neurologic symptoms associated with de novo expansion of the CAG repeat of the TBP gene. Results Case record The patient was a year-old Japanese female. She was the third offspring born to non-consanguineous parents. Her two elder siblings and her parents are healthy. Her birth and development were normal. At the age of 6 years, she was noted to show gait disturbance and intellectual deterioration. At age 9, she showed obvious gait unsteadiness due to truncal ataxia, spasticity and muscle weakness. Furthermore, she had a few episodes of atypical absence at age 9. The symptoms were slowly progressive, and she became confined to a wheelchair at age At age 14, she was thin and short-statured height: On neurological examination, she showed marked cerebellar ataxia of the limbs and the trunk, hyperreflexia, extensor plantar responses, cerebellar dysarthria, difficulty in swallowing and severely impaired intellectual performance. Examination of the optic nerve visual acuities, visual fields and optic fundi , oculomotor nerves and other cranial nerves was normal. There were no sensory disturbances, autonomic dysfunction, involuntary movements or minor congenital anomalies such as high arched palate or polydactylia. Atypical absence was not found after the age of 10 years. Routine laboratory investigations including tests for liver and renal functions were normal. The result of the antibody titer against measles was negative. Examination of the cerebrospinal fluid CSF was normal. Magnetic resonance imaging MRI showed prominent cerebellar atrophy accompanied by dilatation of the fourth ventricle Fig. T1-weighted midsagittal image showed marked cerebellar atrophy accompanied by dilatation of the fourth ventricle. We analyzed the size of the CAG repeats of the TBP gene of 52 patients with autosomal dominant SCAs and eight patients with autosomal dominant epilepsies, for whom expansions of the CAG repeats of previously identified genes 1â€”11 had already been excluded. Furthermore, we also screened 37 patients with sporadic SCAs and 21 patients with sporadic epilepsies. As a result of the screening of the patients, we identified a patient with ataxia, short stature, atypical absence, pyramidal sign and mental deterioration, but without a family history of neurological diseases, who had an expanded CAG repeat coding for 63 glutamines, exceeding the range of CAG repeats in normal individuals 31â€”42 repeats, chromosomes. The expanded allele was observed in the patient but not in her parents or healthy siblings. The patient had an expanded CAG repeat coding for 63 glutamines arrow , which exceeds the range of CAG repeats in normal individuals 31â€”42 repeats, chromosomes. Haplotype analysis of the patient, the siblings and the parents using eight microsatellite markers flanking the TBP gene on chromosome 6q27 revealed that the haplotypes of the patient were clearly inherited from the parents with no evidence of recombination events involving the flanking markers Fig. To

examine whether the gene products of the mutant allele were expressed as a mutant TBP, we performed western blot analysis on the lymphoblastoid cell lines. The signal intensity of the 40 kDa band was comparable with that for the 37 kDa band, suggesting that the mutant TBP is expressed at the level comparable with that of the wild-type TBP Fig. Furthermore, using the 1C2 antibody 19 , which preferentially recognizes large polyglutamine stretches exceeding 40 glutamines, the band corresponding to the 40 kDa mutant protein was more intensely stained compared with that of the 37 kDa protein. Nucleotide sequence analysis of multiple clones demonstrated that the patient has a partially duplicated segment involving the CAG repeats, which encodes 63 glutamines, in addition to the normal allele encoding 36 glutamines. To investigate the subcellular localization of the mutant TBP, we performed immunocytochemical analysis of lymphoblastoid cell lines established from the patient and the mother using an anti-TBP polyclonal antibody. Discussion We identified a patient with ataxia, short stature, atypical absence, pyramidal sign and mental deterioration, but without a family history of neurological diseases, who had an expanded CAG repeat in the TBP gene. The present case with the de novo expansion of the CAG repeat in the TBP gene is the first that involves an impure repeat. The nucleotide sequence and haplotype analyses demonstrated that the mutant TBP gene encoding 63 glutamines derived from a normal paternal allele encoding 39 glutamines. Since the mutation was observed in a non-familial case, the following question would be raised: First, the patient had a CAG repeat encoding 63 glutamines, far exceeding the range in normal individuals [25â€”42 in Caucasians chromosomes 19 and 31â€”42 in Japanese chromosomes Fig. Third, TBP is an important general transcription initiation factor with ubiquitous expression, including in the central nervous system, and the mutant TBP is expressed at levels comparable with those of wild-type TBP and localized in the nuclei of lymphoblastoid cell lines similarly to wild-type TBP. Fourth, from haplotype and nucleotide sequence analysis, it is obvious that the expansion of the CAG repeat in the TBP gene arose as a de novo mutation. Taken together, we concluded that the de novo expansion of the CAG repeat in the TBP gene is most likely to cause the neurological phenotype in the present case. The pathophysiology in this case, however, remains unclear, and may involve toxicity caused by the expanded polyglutamine stretch, as in the other CAG repeat diseases, or impaired transcription function of the mutant TBP. The de novo expansion of the CAG repeat in the TBP gene occurred from the paternal large normal allele, which consists of interrupted CAG repeats, raising the possibility that plural mechanisms exist in the de novo CAG repeat expansion. On the basis of the haplotype analysis Fig. An interesting mechanism can be proposed, based on recent studies in yeast, which showed that mutants lacking the flap endonuclease FEN1 activity demonstrated marked destabilization of CAG repeats that can lead to expansion On the other hand, unequal sister chromatid exchange remains another possibility. These mechanisms may also be involved in the large expansions occasionally observed on paternal transmissions in the other CAG repeat diseases.