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Tiivistelmä Referat Abstract

Cohen syndrome is a disease of the Finnish disease heritage. It is a rare autosomal recessive disorder which is characterized by progressive mental and motor retardation, typical dysmorphic features, microcephaly, retinitis pigmentosa-like ophthalmological changes, granulocytopenia, short stature and hypotonia. The COH1 gene for Cohen syndrome is located on the long arm of chromosome 8 (8q22) and was previously mapped to the close proximity of marker loci D8S1808, D8S1762 and D8S546.

In order to identify the COH1 gene I used the cDNA direct selection method. cDNA direct selection method is based on the hybridization of highly homologous genomic DNA to cDNA. Genomic DNA from BACs 207J1 and 22B10 and PAC 243O19 containing marker D8S1762 were PCR biotinylated and immobilized on streptavidin coated beads. I synthetized double stranded cDNA from human fetal total brain RNA library (Clontech^{R}) and human total skeletal muscle RNA library (Clontech^{R}). Two additional cDNA libraries were used: human adult brain cDNA library (Clontech^{R}) and human fetal retinal library. cDNAs were ligated to adaptors and PCR amplified with primers complementary to adaptor sequences. PCR amplified cDNAs were blocked with Cot-1 to select against highly repetitive sequences. Two cycles of cDNA direct selections were performed with two PCR enrichment steps. Eluted cDNA fragments were cloned directly into a TA-vector. 400 clones were screened for the presence of the cDNA insert using blue-white selection and 2/3 of the clones contained an insert. Southern blotting with ^{32}P labelled whole genomic DNA was performed to select against highly repetitive sequences. I also back hybridize selected, non-repetitive cDNA, clones to the genomic DNA source used in cDNA direct selection. All clones hybridized back, from which I concluded that the cDNA sequences are similar or have very high homology to our genomic DNA. All together 14 cDNAs were selected for sequencing. Sequences were aligned against databases using BLAST search to find homologous DNA sequences. Six cDNA sequences were found to be similar to the mitochondrial and ribosomal DNA and 7 to other genes and genome segments outside the COH1 region.

My results do not indicate the sequence of COH1 gene. Instead the results suggest the idea that the COH1 region on the chromosome 8q22 is highly rearranged and contain the large amount of repetititive sequences.

Avainsanat Nyckelord Keywords
COH1, Cohen Syndrome, cDNA direct selection, Positional cloning

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