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Sequence Refinement of the Human Tyrosinase Clone (Oculocutaneous Albinism OCA 1A)

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Human tyrosinase is often described. Mainly the mutant form, contributing to different forms of albinism appears in literature. This mutant results in a total or parcial lack of color in skin, eyes and hair, occuring among many species. This form of tyrosinase is non-functional. Normal human tyrosinase shows phenole oxidase activity and converts tyrosine to L-3,4-dihydroxyphenyl-alanine (L-DOPA) and further to dopachrome and is a part of the melanin biosynthesis pathway. There has been a sequence published of the oculocutaneous albinism (OCA 1A) form of human tyrosinase. But the correct sequence is often not known because of base insertions, deletions or single nucleotide polymorphisms (SNPs). This paper wants to contribute to the research about that topic and provides the new and corrected sequence of the human tyrosinase clone of oculocutaneous albinism (OCA 1A).

Keywords: Corrected sequencing, Human tyrosinase, Oculocutaneous albinism, OCA 1A

INTRODUCTION

Oculocutaneous albinism occurs due to a total or partial deficiency of tyrosinase activity. An overview is given in literature exactly describing the phenotype [1]. The disease is related to mutations both inside and outside the coding region of the gene [2]. The cytogenetic locus of the human tyrosinase gene is: 11q14-q21, which is the longer (q-)arm of chromosome 11 between bands 14 and 21. Even temperature sensitive mutants are known [3]. Cloning of human tyrosinase, resulting in this form of albinism is often reported and it's sequence has been described [4]. A putative tyrosinase sequences was also found in the past [5]. The exact sequence of the gene is required, because of the reading frame for expression of the recombinant protein. Single nucleotide polymorphisms only affect the protein sequence, but nucleotide deletions [6] or insertions also affect the reading frame. This article wants to contribute to this kind of sequence mismatch of the human tyrosinase gene (oculocutaneous albinism 1A) and provide a new and revised sequence of human tyrosinase (occulocutaneous albinism 1A).

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EXPERIMENTAL

The vector DNA pCMV-XL5, containing the tyrosinase gene (oculocutaneous albinism 1A; BC027179) was from Origene. A plasmid purification kit from Qiagen (Plasmid Midi Kit; Cat. No. 12143) was used. The restriction digest was done by the restriction enzymes EcoR I, Not I and Dra I. All restriction enzymes, the DNA marker (Gene Ruler 1 Kb DNA ladder; Cat. No. SM0311) and agarose were from Cinnagen Co. The buffers and conditions of the manufacturer were used.

Agarose gel electrophoresis was performed with 1x TAE (pH 8.3) and 1 % agarose at 70 mA during 1.5 h. Tris-base and acetic acid was from Aldrich. All other reagents were the highest available purity. Sequencing was done by the didesoxy sequencing method with fluorescent didesoxy nucleotide triphosphates by Kawsar Biotech Co. (KBC), using the following primers: forward GGACTTTCCAAAATGTCG; reverse ATTAGGACAAGGCTGGTGGG. The sequencing was done with a capillary-based method with the BigDye Cycle Sequencing Kit from ABI on a ABI 3130 apparatus (96 reactions) [7]. The clone of oculocutaneous albinism (OCA 1A) was produced in the e.coli strain DH5α, using the ampicillin resistance and purified using a plasmid midi kit.

RESULTS AND DISCUSSION

Competent e.coli cells were prepared by the rubidium chloride method. The restriction digests show the fragments, created by the enzymes. Different enzymes were used for cutting of the DNA. The Not I digest excises the reading frame at two sites up and downstream of the start- and stop codons. The EcoR I digestion linearizes the plasmid and the sequence contains one EcoR I site.

The digest shows, that EcoR I linearizes the plasmid and cuts once. Not I cuts twice and yields two bands. By the restriction digest, an EcoR I restriction site was found in the clone, which was not mentioned in literature. Dra I as a comparison produced four fragments. The resulting sequence was 1665 bp in length and contained the tyrosinase open reading frame (ORF) from bp 200 to bp 1333, starting with the start codon ATG (underlined). The stop codon is TGA (underlined) starting at position 1331. This sequence contains an EcoR I site and is cut at position 106/110 of the given sequence (bold). This is caused by an additional dA-nucleotide compared to the sequence published elsewhere [8]. The dA-nucleotide is within the 5‘-untranslated region (5‘-UTR). This causes a shift of the reading frame, when subcloning the insert into an expression vector, which contains the first ATG after the promotor. Checking of sequences for restriction sites and reading frames was done with NEB-cutter [9]. The complete sequence of the insert is given below:

TCACAAAACAGGGGGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACC
GTCAGAATTTTGTAATACGACTCACTATAGGGCGGCCGC**GAATTC**CCGGGATATCGTCGACCCACGC
GTCCGAAGAGAAATCTGTGACTCCAATTAGCCAGTTCCTGCAGACCTTGTGAGGACTAGAGGAAGAA
TGCTCCTGGCTGTTTTGTACTGCCTGCTGTGGAGTTTCCAGACCTCCGCTGGCCATTTCCCTAGAGCCT
GTGTCTCCTCTAAGAACCTGATGGAGAAGGAATGCTGTCCACCGTGGAGCGGGGACAGGAGTCCCTG
TGGCCAGCTTTCAGGCAGAGGTTCCTGTCAGAATATCCTTCTGTCCAATGCACCACTTGGGCCTCAAT
TTCCCTTCACAGGGGTGGATGACCGGGAGTCGTGGCCTTCCGTCTTTTATAATAGGACCTGCCAGTGC
TCTGGCAACTTCATGGGATTCAACTGTGGAAACTGCAAGTTTGGCTTTTGGGGACCAAACTGCACAG
AGAGACGACTCTTGGTGAGAAGAAACATCTTCGATTTGAGTGCCCCAGAGAAGGACAAATTTTTTGC
CTACCTCACTTTAGCAAAGCATACCATCAGCTCAGACTATGTCATCCCCATAGGGACCTATGGCCAA
ATGAAAAATGGATCAACACCCATGTTTAACGACATCAATATTTATGACCTCTTTGTCTGGATGCATTA
TTATGTGTCAATGGATGCACTGCTTGGGGGATCTGAAATCTGGAGAGACATTGATTTTGCCCATGAA
GCACCAGCTTTTCTGCCTTGGCATAGACTCTTCTTGTTGCGGTGGGAACAAGAAATCCAGAAGNTGA
CAGGAGATGAAAACTTCACTATTCCATATTGGGACNGGCGGGATGCAGAAAAGTGTGACATTTGCAC
AGATGAGTACATGGGAGGTCAGCACCCCACAAATCCTAACTTACTCAGCCCAGCATCATTCTTCTCC
TCTTGGCAGATTGTCTGTAGCCGATTGGAGGAGTACAACAGCCATCAGTCTTTATGCAATGGAACGC
CCGAGGGACCTTTACGGCGTAATCCTGGAAACCATGACAAATCCAGAACCCCAAGGCTCCCCTCTTC
AGCTGATGTAGAATTTTGCCTGAGTTTGACCCAATATGAATCTGGTTCCATGGATAAAGCTGCCAATT
TCAGCTTTAGAAATACACTGGAAGAGATGGGATTTNTCCATGTTGGCTGGGCTGGTNTCAAACTCCT
GACCTCAAGAGATCCNCCNCCTTGGCCTCCCAAAATGCTGGGATTACAGGCTTGAGCCACCACNCCT
GGCCTAACTTCAAGGTATCAAAGACAAATTAACAATGTNTGATGCATACATAGGACTGCTTTGTAAT
NTTNTACACTAATGATTTGTACTATTTTGGTTTCAGAATCCATTTATAACTNTTACAATATATGAAAA
ATTTNTGCCAGCTTTTATTTATGTGGGTTATATTTATGACTACTTNTCATTTGTAAAACTGAAATAAA
AANATNTTGCATATTTGTATTAATTTAAAAAAAAAAAAAAAAGGGCGGCCGCGGTCATAGCTGTTTC
CTGAACAGATCCCGGGTGGCATCCCTGTGACCCCTCCCCANNCNNNNNNN

Scheme 1

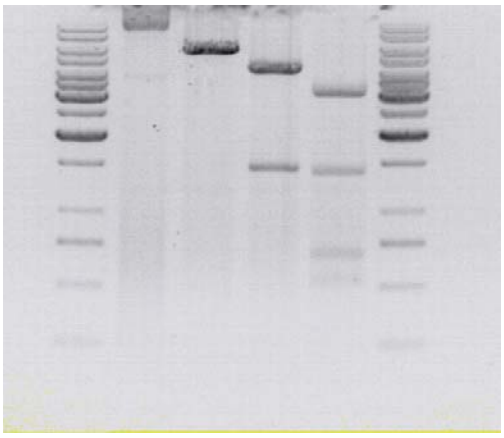

Fig. 1. Lanes 1,6 DNA marker (kbp) 10, 8, 6, 5, 4, 3.5, 3, 2.5, 2, 1.5, 1, 0.75, 0.5, 0.25; lane 2 undigested plasmid; lane 3 EcoR I digest; lane 4 Not I digest; lane 5 Dra I digest.

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REFERENCES

[1] R.A. King, J. Pietsch, J.P. Fryer, S. Savage, M.J. Brott, I. Russell-Eggitt, C.G. Summers, W.S. Oetting, Genetics 113 (2003) 502.

[2] O. Carmand, D. Marchant, S. Boutboul, M. Péquignot, S. Odent, H. Dollfus, J. Sutherland, A. Levin, M. Menasche, C. Marsac, J.L. Dufier, E. Heon, M. Abitbol, Human Mutation 17 (2001) 352.

[3] L.B. Giebel, R.K. Tripathi, R.A. King, R.A. Spritz, J. Clin. Invest. 87 (1990) 1119.

[4] B.S. Kwon, A.K. Haq, S.H. Pomerantz, R. Halaban, Proc. Natl. Acad. Sci. USA. 84 (1987) 7473.

[5] A. Takeda, J. Matsunaga, Y. Tomita, H. Tagami, S. Shibahara, Tohoku J. Exp. Med. 163 (1991).

[6] G. Stevens, J. van Beukering, T. Jenkins, M. Ramsay, Am. J. Hum. Genet. 56 (1995) 586.

[7] F. Sanger, S. Micklen, A.R. Coulson, Proc. Natl. Acad. Sci. USA 74 (1977) 5463.

[8] R.L. Strausberg, E.A. Feingold, L.H. Grouse, J.G. Derge, R.D. Klausner, F.S. Collins, Proc. Natl. Acad. Sci. USA 99 (2002) 16899.

[9] T. Vincze, J. Posfai, R.J. Roberts, Nucl. Acids Res. 31 (2003) 3688.