行政院國家科學委員會專題研究計畫成果報告

中國人第一型白化症的基因突變分析
Mutation analysis of type IA oculocutaneous albinism in Chinese

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主持人：蔡輔仁　　中國醫藥學院醫學系

† 八十六年度及以前的一般國科會專題計畫(不含產學合作研究計畫)亦可選擇適用，惟較特殊的計畫如國科會規劃案等，請先洽得國科會各學術處同意。

一、中文摘要

白化症是自體隱性遺傳疾病，估計其發生率約為四萬分之一。白化症可依其酪胺酸酶活性之有無，分為兩型，其中第一型白化症就是缺乏酪胺酸酶導致。在皮膚、毛髮以及眼睛中的黑色素細胞無法產生黑色素。酪胺酸的基因位於染色體 11q14-21，總共有五個 exons，其 DNA 序列已早為吾人熟悉。白化症患者因其皮膚缺乏色素保護而易導致皮膚癌，而眼睛也會因色素缺乏而有弱視及畏光的症狀。由於各人種的突變位置皆不同，且一直沒有中國人的突變資料，本研究即在此動機下，利用基因定序分析 12 名國人第一型白化症患者基因的突變。在 24 個染色體中總共偵測出 21 個突變點，測出率為 87.5%，其中包括 1 個 splicing site，3 個 insertion/deletion 和 5 個 missense 突變，其中 splicing site 及 insertion/deletion 的突變是未報告過的新突變。232insGGG 這個新突變在國人的發生率極高，佔了 25%之多。本研究不僅偵測出國人白化症的突變點與其他族人有不同之處，亦可提供臨床醫學在產前診斷上的輔助。

關鍵詞：白化症、酪胺酸酶無活性、突變

Abstract

Type I oculocutaneous albinism (OCA1) is an autosomal recessive disorder, which is caused by the reduction or the absence of tyrosinase activity in melanocytes of the skin, hair and eyes. Although mutation study of OCA1 has been extensively studied in most populations worldwide, there is no systemic study of OCA1 mutation in Chinese patients. By use of single strand conformation polymorphism and direct sequencing, we had detected 21 mutant alleles out of 24 OCA1 chromosomes screened (87.5%). Detected mutant alleles include one splicing site, three insertion/deletion and five missense mutations, of which the splicing site nucleotide alteration and two each of the insertion/deletion and missense mutations are novel. The ins/del mutations accounts for about 37.5% in Chinese OCA1 alleles. The 232insGGG, one of the novel mutations, was found to be most frequent (25%) among the OCA1 alleles in Chinese. Through this study, we found that while some of the OCA mutant alleles were identified in other populations, ethnic difference still exists.

Keywords: oculocutaneous albinism, mutation analysis, Chinese

Introduction

Oculocutaneous albinism (OCA), with an estimated frequency of 1: 20,000 in most populations, is the most common inherited disorder of generalized hypopigmentation. It is characterized by reduced or absent biosynthesis of melanin pigment in melanocytes of the skin, hair follicle, and eyes (King and Summers, 1988). OCA can be separated into three general types: type I, type II, and type III OCA. Type I (OCA1)

results from deficient or absent activity of melanocyte tyrosinase (E.C.1.14.18.1), a copper-containing enzyme that catalyzes the first two steps in the melanin biosynthetic pathway (Lerner and Fitzpatrick, 1950). Both type II and III OCAs have normal tyrosinase activity and their genetic abnormalities involve P protein gene (Lee et al., 1994) and tyrosinase related protein (Boissy et al., 1996), respectively. The human tyrosinase locus (TYR) has been mapped to chromosome 11q14-21 by *in situ* hybridization (Barton et al., 1998). TYR gene contains 5 exons spanning more than 65- kb with exon size ranging from 819-bp in exon 1 to 148-bp in exon 3 (Giebel et al., 1991).

Type I OCA is further divided into two subtypes: type IA and IB. In classic, type IA OCA tyrosinase activity and melanin biosynthesis are completely absent and affected patients present with the albino phenotype of white hair and skin throughout life. In type IB ("yellow") OCA the tyrosinase activity and melanin biosynthesis is greatly reduced. Type IB OCA was first described in inbred Amish kindred and was later found in other populations (Nance et al., 1970). The identification of apparent type IA/IB compound heterozygotes indicated that type IA and IB were allelic (Hu et al., 1980; Giebet et al., 1990). To date, at least 66 different pathologic mutations of the tyrosinase gene have been reported in patients with type I OCA (Spritz RA, 1980; Oetting and King, 1993; Tripathi et al., 1993; Gershoui-Baruch et al., 1994).

Although mutation analysis of OCA1 has been extensively studied worldwide, similar studies had never been reported in Chinese OCA1 patients. By use of single strand conformation polymorphism (SSCP) and direct sequencing, we detected 21 mutant alleles out of 24 Chinese OCA1 chromosomes studied.

Materials and methods

Subjects

DNA was isolated from peripheral blood collected from 12 unrelated patients, by use of a DNA Extractor WB kit (Wako, Tokyo, Japan). All patients were characterized with features of OCA1, including white skin and hair, translucent irides, nystagmus, photophobia and reduced visual acuity.

SSCP analysis and DNA sequencing

Exons 1-5 of tyrosinase were PCR amplified and were subjected to mutation analysis by SSCP using a GenePhor DNA Electrophoresis System (Amersham Pharmacia, Quarry Bay, Hong Kong). PCR amplification primers used were listed in Table 1. The PCR products were first diluted with a sample buffer (10 mM Tris, 1 mM EDTA, xylene cyanol 0.05%, bromophenol blue 0.04%, adjusted to pH 7.5 with acetic acid) to about 2 ng/μl and then denatured 1:1 in a denaturing solution (formamide 94%, xylene cyanol 0.05%, and bromophenol blue 0.04%) at 95℃ for 5 min and thereafter directly placed on ice to prevent reannealing of the single stranded products. The samples were then applied to a GeneGel Exel 12.5/24 kit (Amersham Pharmacia, Quarry Bay, Hong Kong) following the manufacturer's recommendation. Exons that exhibited irregular shift by SSCP were subjected to direct sequencing for mutation identification. Before direct sequencing, PCR fragments were purified from agarose gel using QIAEX II (Qiagen, Hilden, Germany). Direct sequencing was performed using a dRhodamine DyeDeoxy Terminator Sequencing kit (PE Applied Biosystems, Foster City, CA) with an ABI Prism 377

DNA Sequencer (PE Applied Biosystems).

Polymorphism analysis

All novel mutations detected in this study were analyzed in 100 normal controls to exclude the possibility of polymorphism. To determine the allele status of 232insGGG, gene scan analysis was utilized. TYR exon 1 covering nucleotide 232 was PCR amplified with primers OCA867(+): 5'-GCCTCAATTTCCCTTCACAG-3' and ex1a(-). The PCR amplification condition was set as following: one cycle at 94℃ for 2 min, 25 cycles of 94℃ for 15 s, 48℃ for 20 s, and 72℃ for 30 s, and one final cycle of extension at 72℃ for 30 m. Standard reaction buffer was used in the PCR amplification except 0.5 μM R110 (fluorescent rhodamine dye)-labeled dUTP (blue) was added into the reaction mixture. The reaction mixtures were run on the ABI Prism 377 DNA Sequencer and GeneScan-350 TAMRA (yellow) (PE Applied Biosystems, Foster City, CA) was run at the same time as an internal molecular weight standard. Collected data was analyzed using software GeneScan Analysis 2.1 (PE Applied Biosystems, Foster City, CA).

Results

Mutation analysis

By use of SSCP and direct sequencing, we detected 21 mutant alleles out of twelve OCA1 patients. Detected mutant alleles include one splicing-site, three insertion/deletion (ins/del), and five missense mutations (Table 2). Three of the missense mutations (Cys55Tyr, Arg299His, and Arg299Ser) were reported in Caucasians (King et al., 1991; Tripathi et al., 1992; Spritz et al., 1997) and one of the ins/del mutations (926insC) was reported in Japanese and Korean (Tomita et al., 1989; Park et al., 1997). Five novel mutations include the splicing-site alteration (IVS1-3C →G), two missenses (Cys288Gly and Trp400Leu), and two ins/dels (232insGGG and 862delTT). Figure 1 shows the electropherograms of direct sequencing result of those novel mutations. One of the probands was found to have, in addition to a 2-bp deletion (862delTT) mutation, two different missense mutations at the same residue (Arg299His and Arg299Ser). Mutation analysis of the proband's parents indicated that he inherited the Arg299Ser allele from his father and the other allele with both 862delTT and Arg299His from his mother.

Polymorphism exclusion of 232insGGG using GeneScan analysis

All those novel mutations detected in this study were screened in normal population for polymorphism exclusion. To verify that 232insGGG is indeed mutation rather than polymorphism, exon 1 region covering nucleotide 232 was amplified and analyzed as described in the Materials and Methods. A normal allele would display a 197-bp band, while the 232insGGG allele would display a 200-bp band (data not shown). In 100 normal controls, all displayed one 197-bp band while the probands with 232insGGG homozygous or 232insGGG heterozygous displayed one band (200-bp) or two bands (197-bp and 200-bp), respectively. From the GeneScan analysis described above, we verified that the 232insGGG allele is indeed mutation rather than polymorphism.

Discussion

In this study, we found 21 OCA1

mutation alleles out of 24 chromosomes and identified 5 novel mutations. Among those five novel mutations, both missense substitutions are non-conservative and the residues involved (cysteine 288 and tryptophan 400) are both conserved between mouse and human tyrosinase polypeptides. It was reported that most of TYR mutations were clustered in three regions of the tyrosinase polypeptide. Two of them (codons 206-278 and codons 355-406) were the copper binding site and amino acid substitution in this region might disrupt copper binding of this polypeptide. The third one is located near the N-terminal of the mature polypeptide and mutation in that region might interfere with the folding of the polypeptide (Tripathi et al., 1992). Although cysteine 288 is not located within the first cluster (codons 206-278), it might either act as a copper binding ligand or function through disulfide bond maintaining normal tertiary structure for the first copper binding region. Replacing it with glycine residue might either eliminate the copper binding ligand or disrupt the tertiary structure of the first copper-binding cluster, respectively. Tryptophan 400 is located in the second cluster, and replacing it with non-conservative leucine residue might disrupt the second copper binding site of this polypeptide.

The 862delTT, one of the novel ins/del mutations, causes the frame-shift of the open reading frame and introduces a premature termination codon (TAA) at codon 299, resulting in a truncated polypeptide. 232insGGG, the other novel ins/del mutation, is in-frame, which introduces a glycine residue between arginine 77 and glutamic acid 78 in the regular reading frame. The IVS1-3C →G would cause aberrant splicing of TYR messenger RNA. Although functional studies were not performed to confirm the detrimental effects of 232insGGG, 232 IVS1-3C→G and both missense mutations, normal population screening (n=100) found none of the aforementioned alleles among normal chromosomes.

The T373K and P81L mutations, which combined together were observed 25-30% in northern Europe (Spritz, 1993; Spritz et al., 1997), were not found in Chinese OCA1 chromosomes. G47D allele, which is common in the Morocan Jews (Gershoni-Baruch et al., 1994) and Puerto Rican (Oetting et al., 1993), was not found either. While three of the missense mutations identified in this study were reported previously in Caucasians, the 926insC allele was reported in both Japanese and Korean (Tomita et al., 1989; Park et al., 1997), with allele frequency as high as 0.5 in Korean OCA1 chromosomes. The 862delTT, while novel, has two thymidine nucleotides deleted at the same nucleotide position as the L288delT allele reported in one Korean OCA1 patient, except two instead of one thymidine nucleotides were deleted (Park et al., 1997). The ins/del mutations accounted for about 37.5% in Chinese OCA1 alleles. The 232insGGG allele, one of the ins/del mutations, is most frequently observed in Chinese OCA1 chromosomes, accounting for 25%. From this study, it seemed that while some OCA1 mutant alleles were shared by different populations, ethnic difference in the spectrum of OCA1 mutation still exists. Mutation analysis of OCA1 in Chinese was first systemically performed in this study. The detection rate is relatively high. Three of the twelve OCA1 patients were found with only one OCA1 mutant allele. The OCA1 mutant alleles that were not identified might be small deletions, which were not identified using the aforementioned method. OCA1 mutations might also be present in the

promoter region or the intron region far away from the intron/exon junction site, which were not investigated in this study. Mutation data obtained in this study will be helpful for future genetic counseling and prenatal diagnosis of OCA1.

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Table 1. Primers used for amplification of TYR Gene Exons by PCR

Primer	Sequence	Position[a]	Sequence amplified
ex1a(+)	5'-TGCTGGAGGTGGGAGTGGTA-3'	538-557	exon 1 5' adjacent region plus exon 1
ex1a(-)	5'-CCTTCTCTGGGGCACTCAAA-3'	1063-1044	5' portion
ex1b(+)	5'-CTTCATGGGATTCAACTGTGG-3'	951-971	exon 1 3' portion plus exon 1 3'
ex1b(-)	5'-TACCCTGCCTGAAGAAGTGAT-3'	1557-1537	adjacent region
ex2(+)	5'-CTACTGACTGGTGGTGACAAT-3'	1693-1713	exon 2 and its flanking region
ex2(-)	5'-TTAAAAGTGAAAAGAAAGAGA-3'	1984-1964	
ex3(+)	5'-GGGTATCCAGAATGTAAAGA-3'	2128-2146	exon 3 and its flanking region
ex3(-)	5'-AAATCCAATGAGCACGTTAT-3'	2479-2760	
ex4(+)	5'-TATGCCTTATTTTACTTTA-3'	2513-2531	exon 4 and its flanking region
ex4(-)	5'-GTAACACTAGATTCAGCAA-3'	2824-2806	
ex5(+)	5'-TGGGATGTCTTTTTATTTCAG-3'	3022-3042	exon 5 and its flanking region
ex5(-)	5'-CTTTTTGGCCCTACTCTATTG-3'	3301-3281	

[a]According to sequence published by Giebel et al.(1991).

Table 2. Mutations identified in TYR gene

Mutation	Sequence	Exon	Predicted effect	Frequency of associated TYR chromosomes
Missense				
Cys55Tyr	TGT—TAT	1	Either disrupts disulfide bond or eliminates copper binding ligand	1/24
Cys288Gly*	TGC—GGC	2	Either disrupts disulfide bond or eliminates copper binding ligand	2/24
Arg299His	CGT—CAT	2	Disrupts copper binding	2/24
Arg299Ser	CGT—AGT	2	Disrupts copper binding	3/24
Trp400Leu*	TGG—TTG	4	Disrupts copper binding	3/24
Insertion/deletion				
232insGGG*	GACCGGGGG GAGTCG	1	Insertion of glycine residue between Arg77 and Glu78	6/24
862delTT*	CAGTCTTTA TGCAAT	2	Truncated polypeptide	1/24
926insC	TCCAGAACC CCCAAGGC	2	Truncated polypeptide	2/24
Splicing				
IVS1-3C—G*	acagGATTTG	2	Aberrant splicing	1/24

Note. Mutation sites are underlined. Numbering of base pairs and amino acids begins at the ATG initiation codon published by Kwon et al.(1987). Mutations labeled with * represent novel mutations detected in this study.

Figure 1. Electropherograms showing raw data for the identified novel mutations in TYR gene. All mutant alleles, except IVS1-3C—G, were shown with forward sequencing. (A) **Cys288Gly (TGC—GGC)**, nucleotide sequence demonstrates a T—G transversion, which results an amino acid change of Cys288Gly. (B) **Trp400Leu (TGG—TTG)**, nucleotide sequence demonstrates a G—T transversion, which results an amino acid change of Trp400Leu. (C) **232insGGG**, nucleotide sequence demonstrates the insertion of three guanine nucleotides at nucleotide position 232. (D) **862delTT**, nucleotide sequence demonstrates the deletion of two thymidine nucleotides at nucleotide position 862. (E) **IVS1-3C—G**, nucleotide sequence demonstrates a C—G transversion at the intron 1 -3 nucleotide position. Arrows indicate the positions of the mutation.