대한체질인류학회지 제 29 권 제 3 호
Korean J Phys Anthropol Vol. 29, No. 3 (2016) pp. 93~98
http://dx.doi.org/10.11637/kjpa.2016.29.3.93

Original Article

Reevaluation of Single Nucleotide Polymorphism of *OCA2* in Koreans

Ann-Yae Na[1], Dae-Kwang Kim[1,2]

[1]*Department of Medical Genetics*
[2]*Hanvit Institution for Medical Genetics, Keimyung University*

(Received 16 June 2016, revised 11 September 2016, accepted 13 September 2016)

Abstract : Oculocutaneous albinism type 2 (OCA2) is an autosomal recessive disorder that results from mutations in the P gene, and has approximately 70% function of melanin biosynthesis in the melanocytes. While the overwhelming majority of pigmentation studies have focused on European populations, very little is known about the gene and mechanisms affecting skin lightening in Asian population. The main goal of the study was to test the distribution of three polymorphisms located in a pigmentation candidate gene, *OCA2*, in a sample of individuals of Koreans (N = 250). The genetic markers were selected for polymorphisms that had an allele frequency difference of at least 30% between East Asian populations and European populations. We investigated Minor Allele Frequencies (MAFs) for each of three polymorphisms within *OCA2* and reevaluated the difference of the allele frequency along with populations. MAFs of polymorphisms of *OCA2* were presented the different frequency in Korean samples (SNP rs1800414 (His615Arg), A allele = 38.8%, rs74653330 (Ala481Thr), A allele = 0.8% and rs7497270 (intronic polymorphism), C allele = 33.4%). While our results had different distributions to European and Caucasians, they showed similar frequencies with East Asian. This study was to reevaluate the distribution of pigmentation candidate gene in Korean samples, further domestic study will aid in developments of the genetic information on worldwide study.

Keywords : Koreans, *OCA2*, Single nucleotide polymorphism

Introduction

The last decade has witnessed important advances in our understanding of the genetic basis of normal variation in human pigmentation [1]. To date, approximately 400 genes have been identified as pigmentation candidate genes that contribute to determination of skin color [2]. Among numerous genes, oculocutaneous albinism (OCA) is an autosomal recessive disorder that is characterized by reduced or absent biosynthesis of melanin pigment in melanocytes of the skin, hair follicles, and eyes [3]. Human OCA has several distinctive phenotypes of type 1 (OCA1; MIN #203100), OCA type 2 (OCA2; MIN #203200), OCA type 3 (OCA3; MIN #203290, and OCA4 (MIN #606574) [4]. The most common type OCA1 and OCA2 are caused by homozygous or compound heterozygous mutations in the *OCA1* (tyrosinase gene; *TYR*) and *OCA2* (P) gene), respectively. *OCA1* which is located in chromosome 11q14-q21, is expressed in melanocytes and controls the

*This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MSIP) (No. 2014R1A5A 2010008).
The author(s) declare that there are no conflicts of interest.
Correspondence to : Dae-Kwang Kim (Department of Medical Genetics, School of Medicine, Keimyung University, 1095, Dalgubeol-daero, Dalseo-gu, Daegu 42601, Republic of Korea)
E-mail : dkkimmd@kmu.ac.kr

ⓒ 2016 Korean Association of Physical Anthropologists
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/ licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
ISSN 2287-626X (Online) · ISSN 1225-150X (Print)

major steps in pigment production [3]. Another common type, OCA2 is caused by mutation in the pick eyed dilution gene (P; *OCA2*) [5]. The OCA2 protein is important for normal biogenesis of melanosomes [6], and normal processing and transport of melanosomal proteins such as TYR and tyrosinase-related protein 1 (TYRP1). The prevalence of the various forms of OCA is shown widely in different populations. OCA1 is the common form of albinism, with a prevalence of 1 per 40,000 individuals in most populations [7]. And OCA2 is also common type of albinism, accounted for approximately 50% of OCA worldwide. The frequency of OCA2 is approximately 8% in all OCA patients in Japan [8] and occurs in ~1 of 30,000 Caucasians and in ~1 of 17,000 blacks in the United States, and at a surprisingly higher incidence (~1/1400) in Tanzania [9,10]. This diversity is highly correlated with geographical location, including that environmental factors as well as genetic aspects strongly influence skin color [11].

While the overwhelming majority of studies on human pigmentation have focused on European populations, researches in other population groups have been much more limited [12,13]. In addition, despite a large number of reported OCA, very little is known about the genetic basis of pigmentation gene in Koreans. Thus, we investigate the distributions of specific polymorphisms (rs1800414, rs74653330, and rs7497270) in the region of *OCA2* in Korean. Based on the SNPs of *OCA2* that reported in East Asia, we researched the allele frequencies of three polymorphisms within *OCA2* in normal Koreans. Besides, we would discuss the different variants distribution genetic aspects of *OCA2* among various populations. Our knowledge about the polymorphisms involved in domestic frequencies is still in its infancy, we think this study focused on Korean populations are needed to get a global perspective of the genetic aspects of human pigmentation.

Materials and Methods

1. Material

To determine the genotyped of the *OCA2* gene, genomic DNA samples from 250 healthy individuals were obtained from the Biobank of Dongsan Hospital (Deagu, Korea). These samples were used for experiments after approval of the study by the Institutional Review Board (IRB) of Keimyung University, Daegu, Korea (IRB No. 2015-06-066).

2. Single-strand conformation polymorphism (SSCP)

For SSCP, genomic DNA samples were used to genotype rs1800414, rs74653330, and rs7497270 of *OCA2* located in chromosome 15. PCR was performed in a final reaction volume of 30 μL containing ~50 ng of genomic DNA, 0.5 μM of each primer, 0.2 μM of each dNTP, 1.5 mM of $MgCl_2$, 1×PCR buffer, and 0.01 U/μL Taq DNA polymerase (Toyobo Co., Japan). Amplification was performed with an initial denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. Amplified PCR products were subjected to SSCP analysis. Electrophoresis was performed in 10% polyacrylamide gel with 1×TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.3). Gels were silver stained, dried, and scored manually for SSCP variants [14]. The primer sequences for the polymorphisms of OCA2 are listed in Table 1.

3. DNA sequencing

Purified PCR products were sequenced using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems, USA) on an ABI 3730 Genetic Analyzer, and the raw sequence data was edited manually using Chromas Ver. 2.4. Each of the identified SSCP variants was sequenced

Table 1. Primer sequences for PCR-SSCP and detection assay

Variant	SNP region	Sequences
rs1800414	Exon 1	F: 5′-CCG TCT GTG CAC ACT AAC CT-3′ R: 5′-TAC TTC GAA GGC TGT GCT CC-3′
rs74653330	Exon 14	F: 5′-ATG TGG GCC TTT CAC GAT GT-3′ R: 5′-GGA GGT GTG CGT TTA CTG GA-3′
rs7497270	Intron region position: 152422	F: 5′-GCT GCA GGA GTC AGA AGG TT-3′ R: 5′-TGG GAA CAG GCT CTG AAA CC-3′

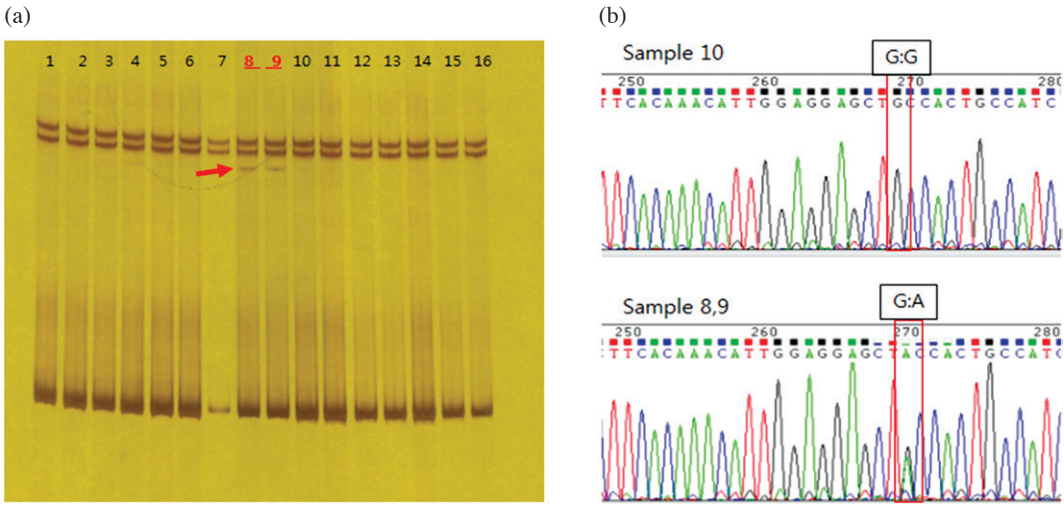

Fig. 1. Analysis of SSCP and DNA sequences for the SNP rs74653330 (Ala481Thr) within *OCA2* in Korean samples. (a) SSCP was performed for the detection of a single-strand DNA fragment by electrophoresis on 10% polyacrylamide gel. (b) DNA sequencing of the SNP (Ala481Thr) region was performed for exon 14 of *OCA2* on chromosome 15.

from both ends using both forward and reverse primers [15]. The exon segments that yielded aberrant patterns were independently reamplified from genomic DNA and sequenced directly.

4. Statistical analysis

The Hardy-Weinberg equilibrium was examined for each variant using the chi-square test. The Hardy-Weinberg equilibrium is a principle stating that the genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors. When mating in random in a large population with no disruptive circumstances, the law predicts that both genotype and allele frequencies will remain constant because they are in equilibrium.

Results

We evaluated the frequency of SNP rs74653330 within pigmentation gene, *OCA2*, in Korean samples. To determine the genotype of the polymorphisms, genomic DNA samples were used for the measurement of SNP frequency by SSCP and DNA sequencing after PCR amplification (Fig. 1). Most of single-strand DNA fragments showed the same pattern by SSCP electrophoresis except 2 among 238 samples (Fig. 1A). The sequence of SNP of *OCA2* in exon 14 predominate G allele approximately 99% in Korean samples and we did not observe any homozygote for the A allele (Fig. 1B).

Table 2 summarized the differences in the distribution of the genotype for the three polymorphism within *OCA2* and frequency in Koreans. The A allele of rs1800414 (His615Arg) was found at a frequency of 38.8%. The other polymorphism located within the *OCA2* gene, rs7497270, showed different allele frequencies that included minor allele frequency (MAF; C allele) 33.4% in Koreans. No significant deviations from Hardy-Weinberg equilibrium were observed for any of polymorphisms.

Discussion

We examined the frequencies of three non-synonymous variants (rs1800414, rs74653330 and rs7497270) within pigmentation candidate gene (*OCA2*) in Koreans. In this study, we observed that the G allele frequency (light skin allele) of rs1800414 was 61.2%, 61 and 105 of 232 subjects were homozygous (G:G) and heterozygous (A:G), respectively. Donnelly et al. reported that rs1800414 (A481T) was almost exclusively in East and Southeast Asia, frequency was at higher levels in eastern East Asia (62~76.1%) compared with Southeast Asia (0~54.3%) and Western China (15.5~37.5%) [16]. Outside of East

Table 2. Genotype frequencies observed for the three polymorphisms analyzed in this study

Gene	Chr (pos)	SNP (alleles)	Genotype frequencies	Allele frequencies	HWE p
OCA2	15 (28197037)	rs1800414 (G/A)	G:G = 61 A:G = 105 A:A = 66	G = 0.612 A = 0.388	0.150
OCA2	15 (28228553)	rs74653330 (G/A)	G:G = 236 A:G = 2 A:A = 0	G = 0.992 A = 0.008	0.948
OCA2	15 (28344328)	rs7497270(T/C)	T:T = 95 C:T = 106 C:C = 35	T = 0.666 C = 0.334	0.543

Chr: chromosome
HWE: Hardy-Weinberg equilibrium
pos: position

and Southeast Asia, the G allele of rs1800414 was only found in low frequencies in Europe (>1~3.6%), Siberia (8.8%), and the Pacific Islands (4.2%). In Japanese, the G allele frequency for A481T reached up to 82% [8,17]. In the Eaton K et al. study [18], the SNP rs1800414 was a nonsynonymous polymorphism of high frequencies (60.2%) in East Asians, which was similar with our data (61.2%). However, this results in Koreans had different level to the distribution of European.

The SNP rs74653330 is another nonsynonymous polymorphism (Ala481Thr) located on exon 14 within the *OCA2* gene. In this study, the frequency of G allele was 99.2% and we did not observe any homozygote for the A allele. Yuasa et al. studied the distribution of this polymorphism in several East Asian populations and found that the frequency of the derived A allele ranged between 0% and 7.4% in Han Chinese and Japanese [17]. The A allele reached much higher frequencies in Mongolia and nearby regions (13% in the Khalha, 51.85% in the Oroqen of Northern China). By contrast, the A allele was not observed in European, South Asian, and African samples [17]. This results indicated that the G allele distribution of rs74653330 in Korean has no different level with East Asian (96.7%) [18], European and Americans (100%) [19].

The other polymorphism located within the *OCA2* gene, rs7497270, showed MAF (C allele) 33.4% in Koreans. Most of European had C allele which the frequency reach 91.4%, American also included 84% with C allele of rs7497270. Among East Asians, the frequency of Japan and Han China included 32.7% and 39.5% [20], these frequencies of East Asian were comparable with our data for C allele.

At first, we planned to investigate the pigmentation candidate polymorphisms both *OCA1* and *OCA2* in Koreans, however, the most of nonsynonymous SNP within *OCA1* has one allele system not only in East Asia but also worldwide. Although this distribution data of SNP according to population was not shown differences within *OCA1*, *OCA2* has different allele frequencies of polymorphism between Western area and East Asian. Therefore we investigated the distribution of polymorphism for pigmentation gene *OCA2* in Korean, because it would be influenced by geographical location, as well as skin color was strongly influenced by environmental and genetic factors.

In summary, the distributions of rs1800414, rs74653330, and rs7497270 within *OCA2* were investigated by selection of an allele frequency difference at least 30% between East Asian populations and European populations. The derived allele of *OCA2* rs1800414 has reached very high frequencies in Korean similar to Chinese, Japanese. On the other side, the derived allele of *OCA2* rs74653330 has a predominant of G allele than rs1800414. The polymorphism rs7497270 of MAF (C allele) has reached 33.4%. Therefore, this study indicated different frequencies for the three variants in *OCA2* had dependency on geographic area, we could suggest that *OCA2* may have a strong effect on the function of melanin level and human pigmentation. Our knowledge about the polymorphisms involved in domestic frequencies is still in its infancy, and further studies focused on Korean populations are needed to get a global perspective of the genetics.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Scheman AJ, Ray DJ, Witkop CJ Jr., Dahl MV. Hereditary mucoepithelial dysplasia. Case report and review of the literature. J Am Acad Dermatol. 1989; 21:351-7.
2. Montoliu L, Gronskov K, Wei AH, Martinez-Garcia M, Fernandez A, Arveiler B, et al. Increasing the complexity: new genes and new types of albinism. Pigment Cell Melanoma Res. 2014; 27:11-8.
3. Oetting WS. Albinism. Curr Opin Pediatr. 1999; 11:565-71.
4. Newton JM, Cohen-Barak O, Hagiwara N, Gardner JM, Davisson MT, King RA, et al. Mutations in the human orthologue of the mouse underwhite gene (uw) underlie a new form of oculocutaneous albinism, OCA4. Am J Hum Genet. 2001; 69:981-8.
5. Rinchik EM, Bultman SJ, Horsthemke B, Lee ST, Strunk KM, Spritz RA, et al. A gene for the mouse pink-eyed dilution locus and for human type II oculocutaneous albinism. Nature. 1993; 361:72-6.
6. Rosemblat S, Sviderskaya EV, Easty DJ, Wilson A, Kwon BS, Bennett DC, et al. Melanosomal defects in melanocytes from mice lacking expression of the pink-eyed dilution gene: correction by culture in the presence of excess tyrosine. Exp Cell Res. 1998; 239:344-52.
7. Biossy RE, Vinson RP, Perry V, James WD, Ortonne JP, Nordlund JJ. Dermatologic manifestations of albinism. Medscape. Available from: http://emedicine.medscape.com/article/1068184-overview#a6
8. Suzuki T, Miyamura Y, Tomita Y. High frequency of the Ala481Thr mutation of the P gene in the Japanese population. Am J Med Genet A. 2003; 118A:402-3.
9. Witkop CJ Jr. Inherited disorders of pigmentation. Clin Dermatol. 1985; 3:70-134.
10. Kromberg JG, Castle D, Zwane EM, Jenkins T. Albinism and skin cancer in Southern Africa. Clin Genet. 1989; 36: 43-52.
11. Parra EJ, Kittles RA, Shriver MD. Implications of correlations between skin color and genetic ancestry for biomedical research. Nat Genet. 2004; 36:S54-60.
12. Edwards M, Bigham A, Tan J, Li S, Gozdzik A, Ross K, et al. Association of the OCA2 polymorphism His615Arg with melanin content in east Asian populations: further evidence of convergent evolution of skin pigmentation. PLoS Genet. 2010; 6:e1000867.
13. Ang KC, Ngu MS, Reid KP, Teh MS, Aida ZS, Koh DX, et al. Skin color variation in Orang Asli tribes of Peninsular Malaysia. PLoS One. 2012; 7:e42752.
14. Ha TW, Han KH, Son DG, Kim SP, Kim DK. Analysis of loss of heterozygosity in Korean patients with keratoacanthoma. J Korean Med Sci. 2005; 20:340-43.
15. Dubey PK, Goyal S, Mishra SK, Yadav AK, Kathiravan P, Arora R, et al. Association analysis of polymorphism in thyroglobulin gene promoter with milk production traits in riverine buffalo (Bubalus bubalis). Meta Gene. 2015; 5: 157-61.
16. Donnelly MP, Paschou P, Grigorenko E, Gurwitz D, Barta C, Lu RB, et al. A global view of the OCA2-HERC2 region and pigmentation. Hum Genet. 2012; 131:683-96.
17. Yuasa I, Harihara S, Jin F, Nishimukai H, Fujihara J, Fukumori Y, et al. Distribution of OCA2*481Thr and OCA2*615Arg, associated with hypopigmentation, in several additional populations. Leg Med (Tokyo). 2011; 13:215-7.
18. Eaton K, Edwards M, Krithika S, Cook G, Norton H, Parra EJ. Association study confirms the role of two OCA2 polymorphisms in normal skin pigmentation variation in East Asian populations. Am J Hum Biol. 2015; 27:520-25.
19. European Bioinformatics institute and the Wellcome Trust Sanger Institute. Ensemble project [Internet]. Hinxton, United Kingdom: [cited 2016 June 1]. Available from: www.ensembl.org, http://asia.ensembl.org/Homo_sapiens/Variation/Populati on?db=core;v=rs74653330;vdb=variation.
20. European Bioinformatics institute and the Wellcome Trust Sanger Institute. Ensemble project [Internet]. Hinxton, United Kingdom: [cited 2016 June 1]. Available from: www.ensembl.org, http://asia.ensembl.org/Homo_sapiens/Variation/Populati on?db=core;v=rs7497270;vdb=variation.

한국인에서 *OCA2*의 단일뉴클레오티드다형태의 재평가

나안예[1], 김대광[1,2]

[1]계명대학교 의과대학 의학유전학교실, [2]한빛의학유전연구소

간추림 : 눈피부 백색증(Oculocutaneous albinism, OCA)은 멜라닌 색소 합성과정의 장애로 피부나 모발, 안구 등 전신에 무색소 또는 저색소증이 나타나는 보통염색체 열성의 이질 유전질환(heterogenous hereditary disorder)이다. 본 연구는 한국인 250명을 대상으로 *OCA2* 유전자에 위치한 3개의 단일뉴클레오티드다형태(Single Nucleotide Polymorphism, SNP)의 분포를 조사하였다. 정상인 DNA를 이용하여 중합효소연쇄반응과 DNA 염기서열분석을 통하여 한국인의 *OCA2* 단일뉴클레오티드다형태의 분포를 확인하였다. *OCA2* 유전자 가운데 단일뉴클레오티드다형태는 동아시아 인구 및 아프리카와 유럽의 인구 사이에 30%의 빈도를 차지하는 것을 기준하여 한국인의 단일뉴클레오티드다형태의 분포와 지역적인 차이를 비교하였다. 한국인에서 *OCA2* 유전자에서 rs1800414과 rs74653330 그리고 rs7497270의 작은대립유전자빈도(Minor allele frequency, MAF)는 각각 A 대립유전자 38.8%, A 대립유전자 0.8% 그리고 C 대립유전자 33.4%로 나타났다. 이 연구 결과는 유럽인과 백인의 빈도와 다르지만 동아시아인에서는 비슷하였다. 국내 *OCA2*의 단일뉴클레오티드다형태에 관련된 유전적 정보가 미비하기에, 한국인에 초점을 맞춘 추후 연구가 세계적인 유전적 정보를 확립하기 위해 유용한 연구가 될 것이다.

찾아보기 낱말 : 한국인, *OCA2*, 단일뉴클레오티드다형태

교신저자 : 김대광(계명대학교 의과대학 의학유전학교실)
전자우편 : dkkimmd@kmu.ac.kr