Research Article

Molecular analysis of families afflicted with autosomal recessive oculocutaneous albinism from Azad Jammu and Kashmir

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Abstract

Autosomal recessive oculocutaneous albinism (OCA) is a heterogeneous group of disorders caused by complete absence or reduction of melanin biosynthesis in the melanocytes. This hypopigmentation can result in mutation due to abnormalities in biosynthetic pathways of melanin. There are six genes which are known for different OCA (OCA1-7). OCA have different types depending upon mutations in different chromosomes. OCA1A is most severe of all types. Present study involves two consanguineous families (A and B), from Jattal, District Mirpur Azad Kashmir. Affected individual were examined for the presence of malformations (other than OCA) and history of metabolic disorders. To identify the causative genes, homozygosity mapping was carried out in both families A and B, by genotyping with microsatellite markers linked to currently known OCA loci. After linkage analysis, it is determined that linkage to OCA1-OCA7 was conclusively excluded in both families "A" and "B". It illustrates that any other identified loci or any probable novel loci may involve.

Key words: Autosomal recessive oculocutaneous albinism; OCA1; OCA7; Linkage Analysis

Introduction

Oculocutaneous albinism (OCA) is an autosomal recessive genetic disorder characterized by partial or complete absence of pigmentation in hair, skin and iris [1]. About 15 genes are associated with different albinism types [2] with 729 mutations linked with any of these loci reported in Human Gene Mutation Database [3]. In Western countries albinism affects 1:17000 newborns

although this frequency ranges from 1:10,000-20,000 worldwide [4, 5] with highest frequency 1 in 1,000 people in Zimbabwe [6]. Albinos have reduced visual acuity, iris translucination, nystagmus, strabismus etc [7].

On the basis of occurrence of mutations, OCA may be syndromic as well as non syndromic. OCA2, TYR, SLC45A2 and TYRP1 are non-syndromic oculocutaneous

albinism genes while *RAB27A*, *HPS1*, *AP3B*, *HPS4*, *BLOC1S3*, *HPS5*, *DTNBP1*, *HPS6*, *LYST*, *MYO5A*, *HPS3*, *PLDN*, and *MLAH* are genes for syndromic OCA [8,9]. Many genes are involved to cause OCA but four main genes are *TYR*, *OCA2*, *TYRP1* and *SLC45A2* [10-13]. Recently two new OCA genes are identified [14]. Additionally, a novel locus of oculocutaneous albinism OCA5 is mapped to 4q24 in a blood related family of Pakistan however, that gene is not described so far [15].

OCA subtypes prevalence differs among different populations. OCA2 is the most frequent form among African and African-American OCA patients [16]. The prevalence of OCA1 is the most frequent type of OCA in Japanese [17], Danes [18], non-Hispanic Caucasians [11].

Materials and methods

Family history

Before the start of this study informed consent was obtained from all family members who participated in the study and the research was approved by Research ethical committee of Mirpur university of Science and Technology. Two families showing autosomal recessive albinism from districts Mirpur of Azad Jammu & Kashmir were recruited for the present study. The Family history was taken and genetic pedigrees (Fig. 1, 4) were drawn according to the standard methods [19].

Fig. 1. Pedigree of family A showing autosomal recessive Oculocutaneous albinism

Blood Sampling

The blood samples were collected from available affected as well as normal individuals of the families (Fig. 2, 5) in ethylene diamine tetra acetate (EDTA) coated tubes having capacity of 10ml, and samples were carried to the laboratory of Human Genetics, Department of Biotechnology, Mirpur University of Science and Technology (MUST), Mirpu-10250, AJ&K. Blood samples were stored at 4°C before DNA extraction.

Fig. 2. Pictures of the affected individuals of family A OCA1

Extraction of genomic DNA from human blood

The extraction of genomic DNA from peripheral blood samples was accomplished by standard phenol-chloroform extraction protocol.

PCR Amplification and Genotyping

PCR amplification of all autosomal markers was performed according to manufacturer specifications.

Microsatellite markers used for genotyping included D15S1506, D15S1002, D15S1019, D9S759, D4S2961, D4S3043, D11S1367, D9S267, D5S5631,

PCR amplification of all autosomal markers was performed according to manufacturer specifications. For genotyping Microsatellite markers used for genotyping included PCR reaction was performed in 0.2 ml tubes (Axygen, USA) containing 25 µl total reaction mixture. The reaction was prepared by adding 1 µl sample DNA (~40 ng), 2.5 µl 10 X buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 1.5 µl MgCl₂ (25 mM), 0.5 µl

dNTPs (10 mM), 0.3 µl of each forward and reverse primer (30 pmole/µl) and 0.2 µl Taq DNA polymerase (5 U/µl, Fermentas, UK) in 18.7 µl PCR water. The reaction mixture was centrifuged for few seconds for thorough mixing. The reaction mixture was taken through thermocycling conditions consisting: 5 minutes at 95°C for denaturation of template DNA followed by 40 cycles of amplification each consisting of 3 steps: one minute at 95°C for denaturing DNA into single strands; 1 minute at 55°C (variable) for primers to hybridize or “anneal” to their complementary sequences on either side of the target sequence; and one minute at 72°C for extension of complementary DNA strands from each primer, final 10 minutes at 72°C for Taq DNA polymerase to synthesize any unextended strands left. PCR was carried out in T1 thermocycler (Biometra, Germany) (Fig. 3, 6).

(A)

(B)

(C)

Fig. 3. Electropherogram of ethidium bromide stained 8% non- denaturing polyacrylamide gel of OCA1 (A-C) showing heterozygosity with Microsatellite marker D15S1019, D4S2961 and D5S1506 Linkage Analysis

In order to find causative gene in four OCA families, pedigrees were analyzed to infer the mode of inheritance. This enabled to create a list of microsatellite markers which can be used to screen families for linkage to known genes and loci responsible for Autosomal Recessive OCA. In both families, search for linkage was carried out by genotyping polymorphic markers mapped within known autosomal recessive OCA loci. Data for all the genotyped loci was analyzed by homozygosity mapping which showed the exclusion of known loci.

Family B

Fig. 4. Pedigree of family B showing autosomal recessive Oculocutaneous albinism

Fig. 5. Pictures of the affected individuals of family B

(A)

1A 2N 3N 4A 5A 6N 7A

(B)

1A 2N 3N 4A 5A 6N 7A

(C)

1A 2N 3N 4A 5A 6N 7A

Fig. 6. Electropherogram of ethidium bromide stained 8% non- denaturing polyacrylamide gel of family B (A-C) showing hetrozygosity with Microsatellite marker D11S1367, D1S1002 and D4S3043

Results

In our study two kashmiri families (A, B) with autosomal recessive Occulocutanious albinisim were ascertained. The pedigree was constructed by interviewing with multiple family members while personal interviews with key figures in the kindred clarified the con-sanguineous relationships. The banding Pattern showed heterozygous bands indicating the exclusion of the causative gene to known loci.

Consanguineous pedigrees provide convincing evidence of autosomal recessive mode of inheritance. Out of two OCA families; no family was linked to known OCA loci. Microsatellite markers selected from the Marshfield genetic map were according to the prevalence of OCA in previous reported families (Table 1, 2).

Table 1. Clinical and morphological features of affected individuals from family A

FEATURES	III: 3	III: 4
Age	23 years	18 years
Sex	Female	Male
Height	5.4 inches	5.7 inches
Hair Colour	Blond	Blond
Iris colour	Blue	Blue
Photophobia	Yes	Yes
Nystagmus	Yes	Yes
Obesity	No	No
Hearing loss	No	No
Cataract History	No	No
High arched palate	Yes	No
Widely Spaced Teeth	Yes	Yes
Pigmented Freckles	Yes	Yes
Coarse Facial Features	No	No

Table 2. Clinical and morphological features of affected individuals from family B

Features	III-1	III-2
Age	39 years	28 years
Sex	Female	Male
Height	5 feet 7 inches	5.6 inches
Hair Colour	White	White
Iris colour	Hazel-gray	Hazel-gray
Photophobia	Yes	Yes
Nystagmus	Yes	Yes
Obesity	Yes	Yes
Hearing loss	No	No
Cataract History	No	No
High arched palate	Yes	Yes
Widely Spaced Teeth	No	No
Pigmented Freckles	No	No
Coarse Facial Features	No	No

Discussion

In past albinism was classified on the basis of phenotype but now-a-days it is explained more precisely with the help of molecular biology. Progress in molecular biology has

made it easier to make a diagnosis of several kinds of albinism on the basis of genetic causation. Albinism is a condition which comprehends diseases in which a disorder of melanin system occur. Mutation on different chromosomes leads to different kinds of albinism [20]. Oculocutaneous albinism (OCA) is main type of albinism and it is a group of autosomal recessive diseases in which amount of melanin becomes decreased or there is a complete lack of it. There are more chances of cancer and other skin problems in oculocutaneous albinism [21].

There are different types of OCA. OCA1A is more harmful kind, it involves a total absence of melanin during whole life span of an individual while OCA1B, OCA2, OCA3 and OCA4 are less severe kinds and show some pigment accretion over time. In different genes mutations occur and it leads to different forms of OCA [22]. Almost, 1/17000 people have one of types of albinism [22]. Symptoms of OCA includes decreased ocular acuity, refractive faults, a degree of color vision defect, hypopigmentation of iris [23].

In present study two consanguineous families of Jatlan District Mirpur are showing autosomal recessive oculocutaneous albinism. By pedigree analysis and family histories, autosomal recessive mode of inheritance was established. Affected individuals of both families show pigmentation in skin hair and eyes. The homozygosity mapping was followed by microsatellite markers. These markers were heterozygous for all affected and normal individuals.

Markers which were taken for OCA are D11S1367, D11S4175, D15S1002, D15S1019, D9S759, D9S267, D5S631, D5S1506, D4S2961, and D4S3043. After PCR based genotyping of microsatellite markers, it concludes that OCA1-OCA7 is excluded in both families “A” and “B”. It is

another loci. It may be possibly novel loci. Mutations help to understand about melanin production disorder and other genetic modes of diseases. It will support to diminish the prevalence of OCA in consanguineous population.

Phenotypically affected members were showing reduced pigment in skin as well as some eyes defects and sensitivity to sunlight, genetic analysis was performed in succeeding steps initially family histories were studied and pedigrees were drawn showing autosomal recessive pattern of inheritance. On the basis of this analysis microsatellite markers were selected and checked for homozygosity later than DNA extraction. Our findings indicated that all individuals were heterozygous for all tested markers which showed exclusion of known loci and directed the research toward testing for another known loci or a novel loci.

Conclusion

By analyzing the results of whole experiment that is genetic analysis of both OCA families by PCR, It is concluded that both families of OCA under study provide an evidence for further research as its involvement of mutation of either another known loci or may be concerned to a novel loci related to Albinism, Because results did not showed linkage with any of the tested microsatellite marker. This information is important towards a more specific perception of hereditary origin of inadequately analyzed genetic diseases like albinism, innovation of advanced prognosis as well as strategies to overcome genetic diseases specially in under developed countries like Pakistan and Azad Kashmir.

Authors' contributions

Conceived and designed the experiments: I Ali & AA Abassi, Performed the experiments: U Niaz, K Ishaq & T Aziz, Analyzed the data: I Ali & AA Abassi, Contributed reagents/ materials/ analysis

tools: R Asghar & MA Hussain, Wrote the paper: I Ali & AA Abassi.

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