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Linkage analysis of genes involved in human hereditary hair loss disorder

Hafeezul Haq[1], Amir Atlas[2], Bilal Abbas[1], Arshad Abbas[3], Nargis Jamila[4], Muhammad Rehman[1], Hameed Ur Rehman[*5], Ali Nawaz[2], Hazrat Usman[2]

[1]*Department of Biotechnology, Kohat University of Science and Technology, KPK, Pakistan*
[2]*Department of Bioinformatics and Biotechnology, International Islamic University, Islamabad, Pakistan*
[3]*Department of Zoology, GPGC, Karak, KP, Pakistan*
[4]*Department of Chemistry Shaheed Benazir Bhutto Women University, Peshawar, Pakistabn*
[5]*Department of Chemistry, Kohat University of Science and Technology, KPK, Pakistan*

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Abstract

Hair can be observed at different levels, its structure and function can be modified by its constituent molecules, especially proteins, but sometimes hair follicles can also be genetically altered. Damage to the hair follicle life cycle affects the structure and morphology of the hair. Hypotrichosis is a heterogeneous group of hereditary offenses that pass from one generation to the next in both dominant and recessive forms. Hypotrichosis manifests itself in both symptomatic and non-syndromic forms, exhibiting a broad phenotype. The phenotype ranges from sparse to completely scalp free. Manes, pubic hairs, eyebrows and eyelashes are also affected to varying degrees in different forms of hair. These hairs are present in different parts of the body's roots, forming organized, multi-layered, regenerative and epidermal appendages called hair follicles. . Any mutation in a gene known to be involved in hair follicle development and hair cycle is a causative agent of hereditary hypotrichosis. So far, 7 sites of non-syndromic autosomal dominant hypotrichosis and 8 sites of non-syndromic autosomal recessive hypotrichosis have been localized on different autosomes. Syndromic forms of hereditary hypotrichosis are known to have varying degrees of abnormality.In this study, a family from the region Karak was described as having a non-syndromic autosomal recessive form. A homozygous mapping study using highly polymorphic microsatellite markers established a linkage between the family and the LPAR6 gene located on chromosomes 13q14.11-21.32.DNA sequence analysis using the LPAR6 gene revealed that G replaced A, at nucleotide 436 (c.436G>A, p.Gly146Arg) was derived from (A) control individuals.

***Corresponding Author:** Hameed Ur Rehman ✉ 03449002451h@gmail.com

Introduction

Hypotrichosis is a condition of abnormal hair patterns that is primarily lost or reduced. It most often occurs in hair growth in areas of the body that usually produce terminal hair [R Nalluri, 2016]Normally, individual hair growth is normal after birth, but shortly thereafter, they experience progressive, gradual loss of hair, limited to the scalp, starting in the middle of the first decade, resulting in a completely loss of hair by the third decade. Some individuals with abnormal hair growth will leave some sparse, small, short scalp hair. Body hair, beards, eyebrows, manes, teeth and nails are usually formed. New hair is usually very thin, short and brittle, and may lack pigmentation[JC Vary ,2015]

Symptoms of hair loss include hair loss in the plaque, usually in a circular pattern, dandruff, skin damage and scar formation. Alopecia areata (mild-medium level) usually manifests in areas of unusual hair loss [K McElwe, (2012)]such as eyebrows, the back of the head or above the ears, areas where male pattern baldness usually does not affect. In male pattern hair loss, shedding and thinning begin to occur at the temple, and the crown and hair may become thinner or fall off. Female hair loss occurs in the frontal and parietal leaves [M Leavitt, (2008)]. The aim of the current project was to find out theLinkage Analysis of Genes Involved in Human Hereditary Hair Loss Disorder.

LPAR6 Gene

Lysophosphatidic acid receptor 6, also known as LPA6, P2RY5 and GPR87, is a protein encoded by the LPAR6 gene in humans [66]. LPAR6 is a G protein-coupled receptor that binds to the lipid signaling moleculelysophosphatidic acid (LPA)[V.Ralevic , 1998)].

The protein encoded by this gene belongs to the G protein coupled receptor family, which is preferentially activated by adenosine and uridine nucleotides. This gene is aligned with the internal intron of the reverse retinoblastoma susceptibility gene [K Yanagida, 2009].

Cytogenetic location

13q14.2, which is the long (q) arm of chromosome 13 at position 14.2

Molecular location

Base pairs 48,400,897 to 48,444,704 on chromosome 13 (Homo sapiens Annotation Release 109, GRCh38.p12) [Z.Azeem, 2008].

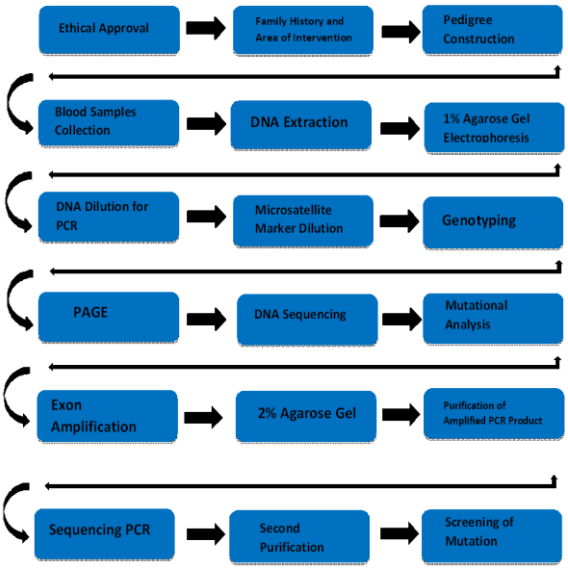
Function of LPAR6 Gene

The LPAR6 gene provides an illustration of the preparation of a protein called lysophosphatidic acid receptor 6 (LPA6). This protein acts as a receptor. Receptor proteins have specific sites in which some other protein (called a ligand) is embedded in the lock like a key. A specific fat called lysophosphatidic acid (LPA) is a ligand for the LPA6 protein. LPA can attach to many receptors, but LPA6 is the only LPA receptor in hair follicles 21. Hair follicles are a special structure for hair growth in the skin. As the cells in the hair follicle divide, the hair line (axis) is pushed up and extends beyond the skin, causing hair growth. LPA6 protein is also present in the outermost layer (skin) of the skin. The attachment of LPA to LPA6 helps regulate hair follicle cell growth and division (proliferation) and maturation (differentiation) [M Kurban2012].

Mutation in the LPAR6 Gene

More than thirty LPAR-6 gene mutations have been found to cause autosomal recessive hypotrichosis, a condition that results in sparse hair growth (hypotrichosis) on the scalp and, less frequently, other parts of the body.Some mutations are specific to people with Pakistani descent[M Kurban2012]. Mutation of the LPAR6 gene results in the production of an aberrant LPA6 protein that is unable to bind to LPA to regulate cell proliferation and differentiation within the hair follicle. As a result, hair follicles are structurally abnormal and are generally underdeveloped. Irregular hair follicles alter the structure and growth of the hair shaft, causing fragile hair to break easily. Lack of LPA6 protein function in the epidermis may lead to skin problems in patients with autosomal recessivehypotrichosis[SM Pasternack, 2008].

Materials and methods



Results and discussion

Family morphology

The selected family belongs to Karak, Kpk, Pakistan. All affected individuals exhibited symptoms of hypotrichosis.

The families participating in this study have three generations and four members. Out of the four members, two males (III-2 and III-3) and one female (III-6) were affected. Clinical examination of affected individuals revealed sparse hair on the scalp. The color of the hair is brownish black.

The eyebrows and eyelashes are very thin and close to missing. No symptoms of any other disease were found. Parents and other normal members of the family did not exhibit a phenotype or analog similar to hypotrichosis.

Linkage and sequence analysis of family

Genotyping results

Blood was collected from four members of the family. The DNA was extracted from all these four members (II-2, III-2, III-3, III-6). Among these members III-2, III-3 and III-6 were affected. Highly polymorphic markers were used for genotyping.

The used markers were D13S287 (51.7 cM) (Fig. 3.3), D13S118 (52 cM) (Fig.3.4), D13S164 (52 cM) (Fig. 3.5), D13S153 (52 cM) (Fig. 3.6), and D13S273 (52.8 cM) (Fig. 3.7). Gel bands study on polyacrylamide gel confirmed linkage of family to LPAR6 gene positioned at chromosome 13q21.3. Sequence analysis disclosed a pathogenic sequence variation at nucleotide position (c.436G>A), substituting Glycine amino acid with Arginine (p. Gly146Arg). Parents of the affected participants remained heterozygous at this position.

Table. Different forms of Autosomal Recessive Hypotrichosis.

Disorder	Locus	Gene	Clinical Features	Reference
Localized Autosomal Recessive Hypotrichosis 1 (LAH1; MIM 607903)	HYPT6 18q12.1	*DSG-4*	Sparse hair on scalp, trunk and Extremities. Normal pubic, axillary and facial hair.	M Farooq, A Zlotogorski, M. Wajid, 2007
Localized Autosomal Recessive Hypotrichosis 2 (LAH2; MIM 604397)	HYPT7 3q27.2	*LIPH*	Low density of scalp and body hair or sparse woolly hair. Few eyebrows and eyelashes.	G. Ali, 2007, S. Khan, 2011
Localized Autosomal Recessive Hypotrichosis 3 (LAH3; MIM 611452)	HYPT8 13q14.2	*LPAR6*	Sparse hair on scalp, trunk and extremities or sparse woolly hair. Few eyebrows and eyelashes.	M.Kurban, 2013, Wali,2007
Autosomal recessive hypotrichosis (HYPT9)	10q11.23	Causative gene unknown	lacking of usual eyelashes, eyebrows, axillary hair and body hair	M. Ayub, 2009, S. Duzenli 2009
Hypotrichosis and	18q12.1	*DSC3*	Nearly devoid of scalp, facial and	J. yang, 2014

Recurrent Skin Vesicles (MIM 613102)			body hair along with thin watery fluid filled vesicles on skin and scalp.	
Hypotrichosis	16q21-q23.1	*CDH3*	partial or complete absence of hair on scalp and on some or all parts of the body.	M. Ayub, 2009, S. Duzenli 2009
Atrichia with Papular Lesions (APL; MIM 209500)	8p21.3	*HR*	No hair on entire body along with cutaneous follicular cysts.	A. A PanteleyevVRalevic , 1998 K Yanagida2009

Discussion

Hypotrichosis is a human hair loss disorder characterized by hair abnormalities caused by defects in the hair growth cycle, hair structure, signaling molecules and pathways involved in hair follicle development. This problem is actually caused by mutations in many genes. To date, 15 different genes have been identified leading to hypotrichosis.

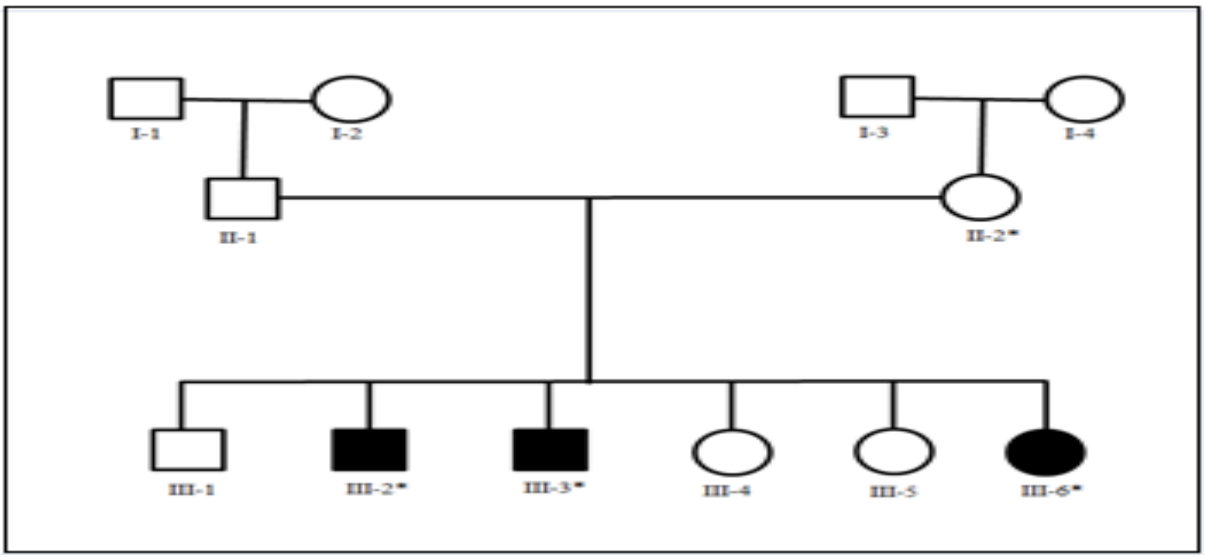


Fig. 1. Pedigree of the family.

Fig. 2. Clinical representation of the two affected members of the family are shown here. A. The affected member III-2 showing complete absence of scalp hair and thin eye lashes and eyebrows. B. Affected member III-3 of family shows retarded hairs on scalp.

The discovery of genes for this disease is a major source of insight into the molecular mechanisms of epidermal development and differentiation. To find out causative gene involved in autosomal recessive hypotrichosis a classical genome analysis method termed as "Homozygosity Mapping" was followed.

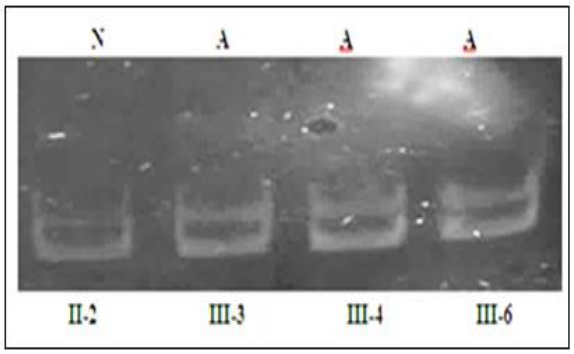


Fig. 3. The Electrophorogram of non-denaturing 8% polyacrylamide gel, stained with ethidium bromide display allele design got with marker D13S287 at 51.70 cM from LPAR6 gene candidate linkage interval at chromosome 13q21.3.

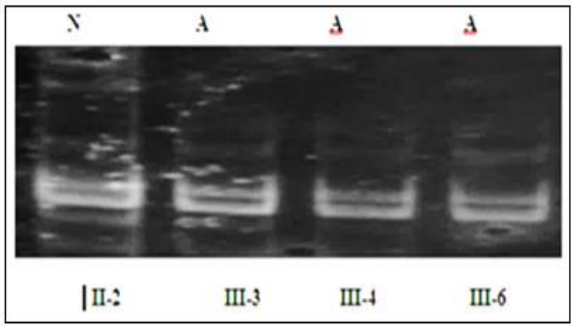


Fig. 4. Electrophorogram of non-denaturing 8% polyacrylamide gel, stained with ethidium bromide display allele design got with marker D13S118 at 52 cM from LPPAR6 candidate linkage interval at chromosome 13q21.3.

Smith in 1953 showed that consanguineous matings giving birth to offspring, will be homozygous for genetic marker near disease gene. Affected offspring of consanguineous marriages have almost homozygous region of few centi Morgan spinning near the affected gene by decent.

Other region may be homozygous by decent but they will vary from one offspring into another within the family.

The homozygosity mapping revolved for identification of homozygous region only in affected offsprings.In the current study, a family from the region Karak was selected.

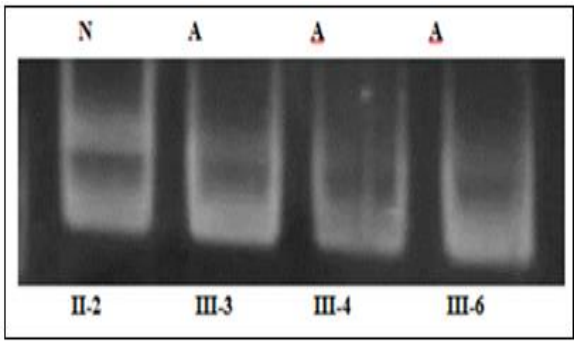


Fig. 5. Electrophorogram of non-denaturing 8% polyacrylamide gel, stained with ethidium bromide display allele design got with marker D13S164 at 52 cM from LPPAR6 gene candidate linkage interval at chromosome 13q21.3.

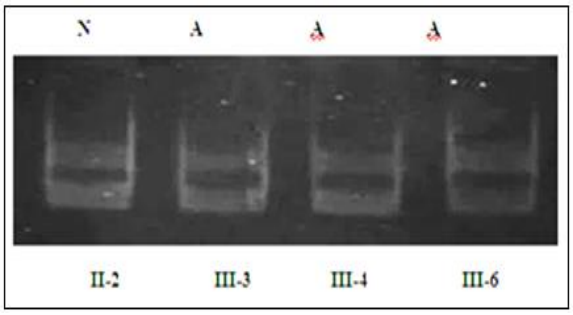


Fig. 6. Electrophorogram of non-denaturing 8% polyacrylamide gel, stained with ethidium bromide display allele design got with marker D13S153 at 52 cM from LPPAR6 gene candidate linkage interval at chromosome 13q21.3.

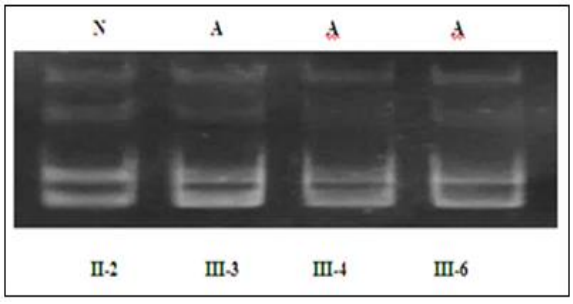


Fig. 7. Electrophorogram of non-denaturing 8% polyacrylamide gel, stained with ethidium bromide display allele design got with marker D13S273 at 52.8 cM from LPPAR6 gene candidate linkage interval at chromosome 13q21.3.

This family exhibits a non-syndromic autosomal recessive inheritance. Clinical data obtained from affected family individuals also support knowledge of non-syndromic autosomal recessive hair loss.

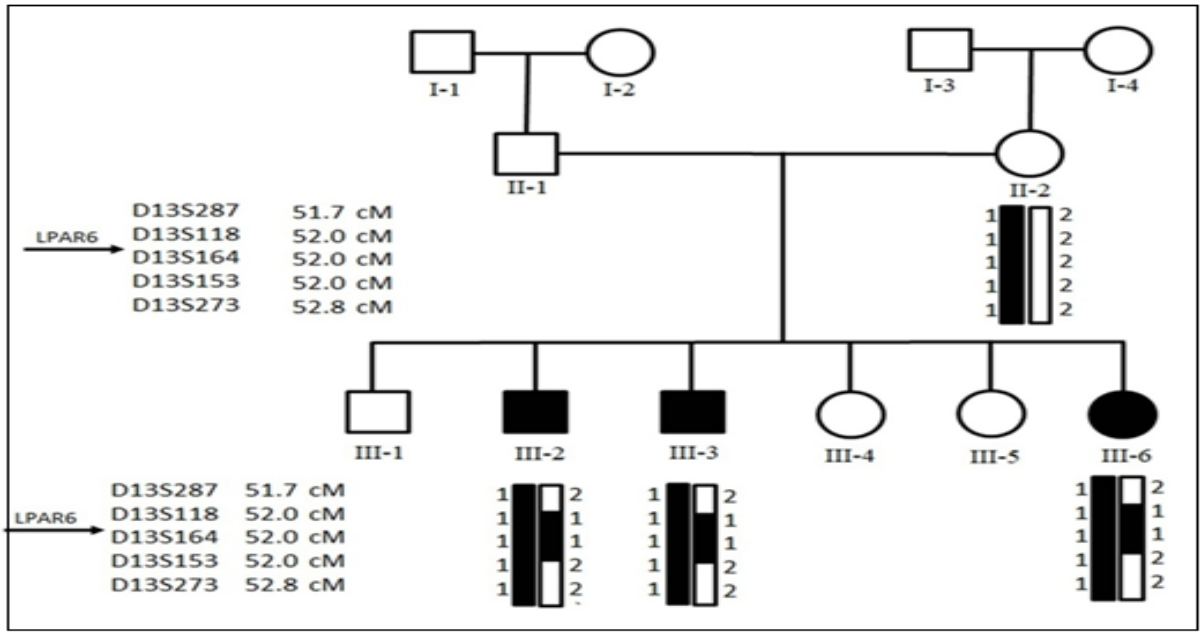


Fig. 8. Haplotype of family.

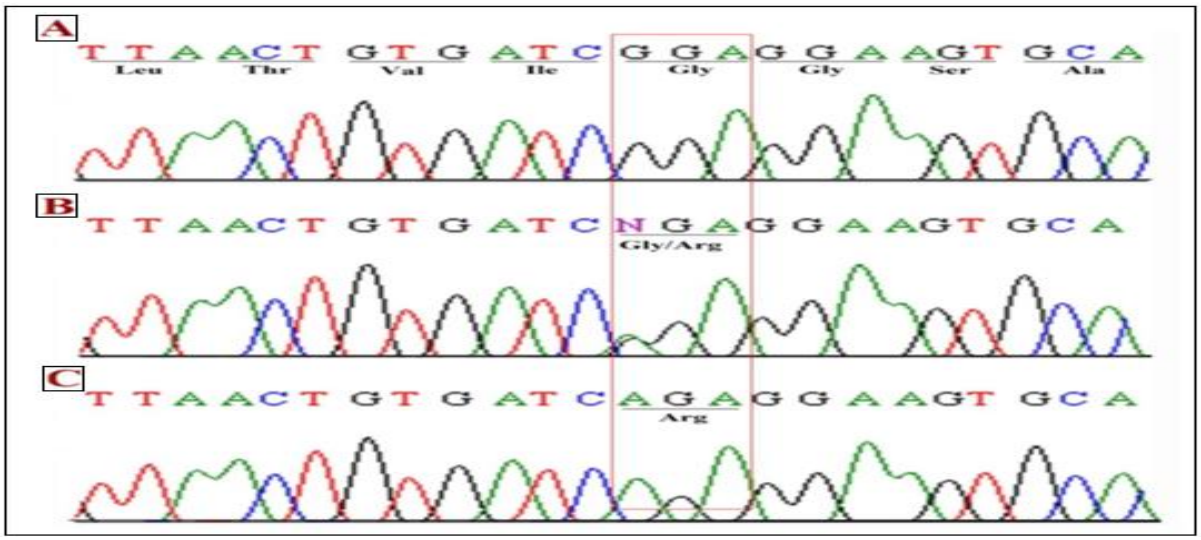


Fig. 9. Sequence analysis of LPAR6 gene in family A. DNA sequence analysis of the LPAR6 gene show a substitution of G with A at nucleotide 436 (c.436G>A, p. Gly146Arg) from (A)a control individual, (B) a heterozygous carrier, and (C) a homozygous affected individual.

The study was conducted in two phases. In the first phase, homozygous mapping of microsatellite markers for genes that cause autosomal recessive hereditary defects (LIPH, LPAR6, and DSG4) was tested. Linkage analysis of microsatellite markers revealed linkage of the family at the locus of the LPAR6 gene. Potential mutations in the LPAR6 gene lead to pathogenic mutations in non-syndromic autosomal recessive hereditary hypotrichosis.

Concluding remarks

From the analysis of the sequencing results, it can be concluded that the identified mutations (c.436G>A, p. Gly146Arg) were identified in this study.

Such reported mutations are the most common and are often found in the population of Khyber Pakhtunkhwa.

The study will help to identify treatment goals, genetic counseling and awareness of issues related to the marriage of close relatives in the Pakistani population.

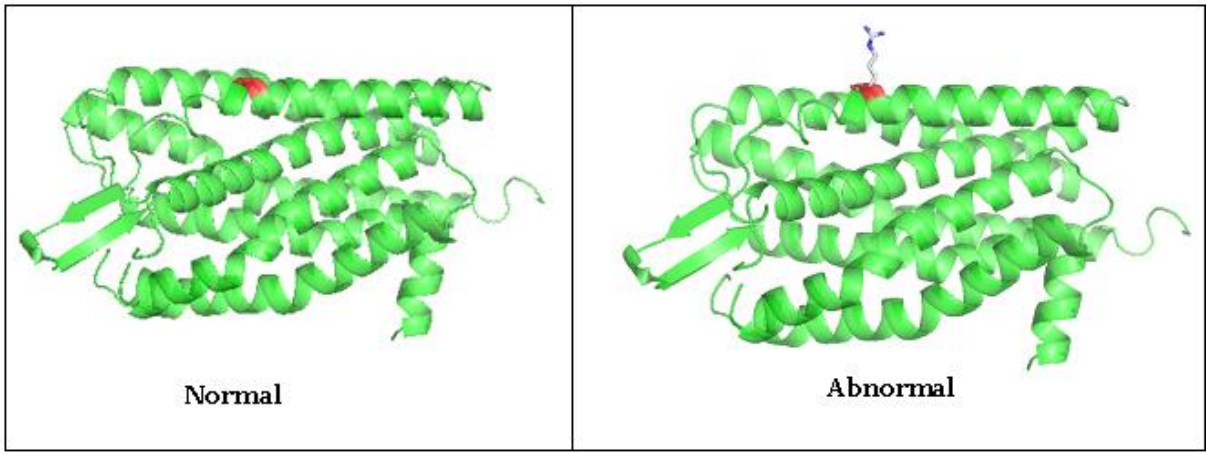


Fig. 10. Structural changes induced by the creating Gly 146 Arg mutation by using PyMOL.

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